Studies of Precursor-directed Biosynthesis with Streptomyces. Part 2.[1] New and Unusual Manumycin Analogues Produced by *Streptomyces parvulus*

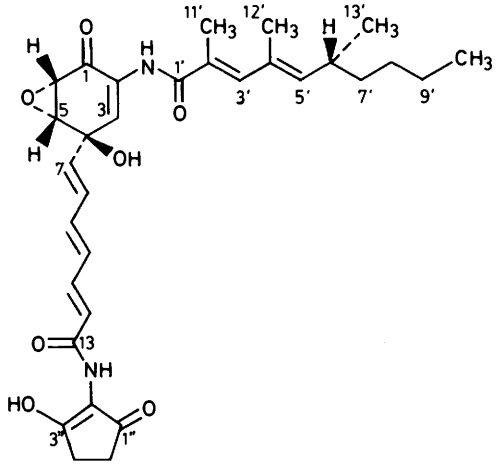
Ralf Thiericke, Hans-Jörg Langer, and Axel Zeeck *
Institut für Organische Chemie, Universität Göttingen, Tammannstrasse 2, D-3400 Göttingen, Federal Republic of Germany

The application of a new type of precursor-directed biosynthesis, in which artificial aromatic starter molecules were fed to the manumycin producer *Streptomyces parvulus*, resulted in new and unusual manumycin analogues for which structures are reported. The new compounds can be divided into three classes; the artificial starter molecule elongated (i) with the triene chain including the C_5N moiety, (ii) with the chiral manumycin C_{13}-side chain only, and (iii) with both substituents. Based on these results it is plausible to differentiate between an amidase linking the chiral C_{13}-side chain to the artificial precursor, and a CoA-transferase activating the aromatic carboxy group for further elongation *via* the polyketide pathway. The specificity of the enzymes involved with regard to the structure of the aromatic precursor is discussed.

The biogenetic origin of the multifunctional *m*-C_7N unit in the manumycin group antibiotics manumycin (**1**) and asukamycin has recently been elucidated.[2–4] In both cases the starter molecule derives from the TCA-cycle intermediate succinate (C_4 unit) and the carbohydrate metabolite glycerol (C_3 unit). Thus, this biosynthetic pathway differs drastically from those described for other antibiotics[5–8] containing a *m*-C_7N moiety. As yet no intermediate has been found on the way from the C_4 and C_3 units to manumycin/asukamycin.

As previously reported,[1] we observed a strong dependence of the metabolite pattern on the amount of 3-aminobenzoic acid (*m*ABA) fed to the manumycin producing strain. A 7 mM *m*ABA solution suppressed the manumycin biosynthesis, while unphysiological concentrations (55 mM) led to a manumycin analogue, called 64-*m*ABA (**2**). It was found that 4-aminobenzoic acid (*p*ABA) led to formation of 64-*p*ABA (**3**), in which the chiral C_{13}-side chain was not linked to the amino group.

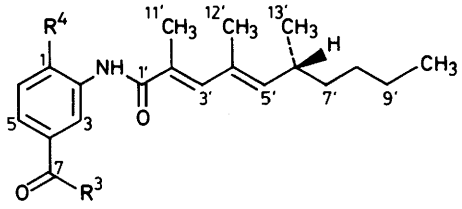
In this paper we describe the extension of this new type of precursor-directed biosynthesis[1] and the replacement of the natural *m*-C_7N unit in the parent antibiotic manumycin (**1**) by unusual artificial compounds in order to obtain new manumycin analogues. We were interested in extending our method to unusual artificial precursors as well as obtaining additional information on the specificity of the enzymes involved in the modification of the artificial and subsequently of the natural precursor in the manumycin biosynthesis.



(**1**)

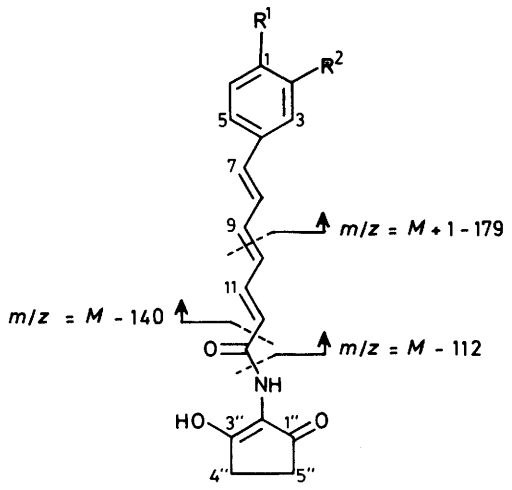
Feeding Experiments and Results

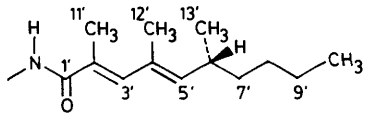
Following our described method[1] we fed a series of different aromatic compounds in unphysiological amounts (55 mM) into the stationary growing phase of *Streptomyces parvulus* (strain Tü 64). The artificial precursors employed included substituted aminobenzoic acids, benzoic acids without an amino functionality, aromatic amines, and non-aromatic compounds. The fermentation conditions and the feeding and the work-up procedures were the same as previously described.[1] The resulting crude products were examined by t.l.c. (silica gel, $CHCl_3$–MeOH, 9:1 v/v) to obtain the metabolite pattern of each feeding experiment in comparison to an unfed fermentation. The appearance of the red C_{25} prodigiosins 36—40 h after inoculation indicates that the secondary metabolism has started and is therefore a conspicuous indicator of normal cell growth. The qualitative estimation of the dye portion in the crude products gave evidence of how strongly the fed substances influence the normal behaviour of the strain used. The results of the feeding experiments are summarized in Tables 1 and 2.



(**8**) R^3 = OH, R^4 = OH
(**9**) R^3 = $NHNH_2$, R^4 = H

After purification the new compounds were characterized spectroscopically, their molecular formulae were determined by high resolution mass spectra, and their structures were elucidated by comparing the ^1H and ^{13}C n.m.r. spectra to those of known manumycin derivatives.[1,9,10] Both, the ^1H and the ^{13}C n.m.r. spectra of (**4**), (**5**), (**6**), and (**7**) are over-crowded in the aromatic/olefinic region. Therefore, the assignments of the signals in this region were made by increment calculations and/or by direct correlation to manumycin (**1**) and 64-*m*ABA (**2**), whose signal assignments were established by two dimensional n.m.r. methods.[1,9] The carbon signals of C-1″, C-3″, C-4″, and C-5″ of compounds possessing the C_5N moiety are known to be broad at 50.3 MHz.[1,9,13] We attribute this effect to tautomeric changes occurring on the n.m.r. time-scale, resulting



	R^1	R^2
(2)	H	
(3)	NH_2	H
(4)	H	OH
(5)	CH_3	NH_2
(6)	OCH_3	NH_2
(7)	OH	OCH_3

Scheme 1. Formulae and e.i.-m.s. (70 eV) fragmentation pattern of class 1 and 3 manumycin analogues

Table 1. Feeding (55 mol l^{-1}) of different artificial aromatic compounds to *Streptomyces parvulus* (strain Tü 64)

Precursor	Manumycin (**1**)	Prodigiosins[a]	New product		R_F Value[b]	Amount (mg l^{-1})
3-Hydroxybenzoic acid	33 mg l^{-1}	Strong	64-HBA	(**4**)	0.10	7.4
Ferulic acid		Very weak	64-VAN	(**7**)	0.39	20.0
Vanillic acid		Very weak	64-VAN	(**7**)	0.39	23.0
3-Amino-4-methylbenzoic acid		Weak	64-3A4M	(**5**)	0.43	12.1
3-Amino-4-methoxybenzoic acid		Weak	64-3A4MO	(**6**)	0.51	8.4
3-Amino-4-hydroxybenzoic acid		Strong	64-3A4Hy	(**8**)	0.33	13.3
3-Aminobenzohydrazide		Weak	64-BZH	(**9**)	0.41	23.0

[a] By observing the colour of the mycelium and the t.l.c. control of the crude products. [b] Silica gel, $CHCl_3$–MeOH (9:1 v/v).

Table 2. Precursor fed to *Streptomyces parvulus* without the appearance of new metabolites

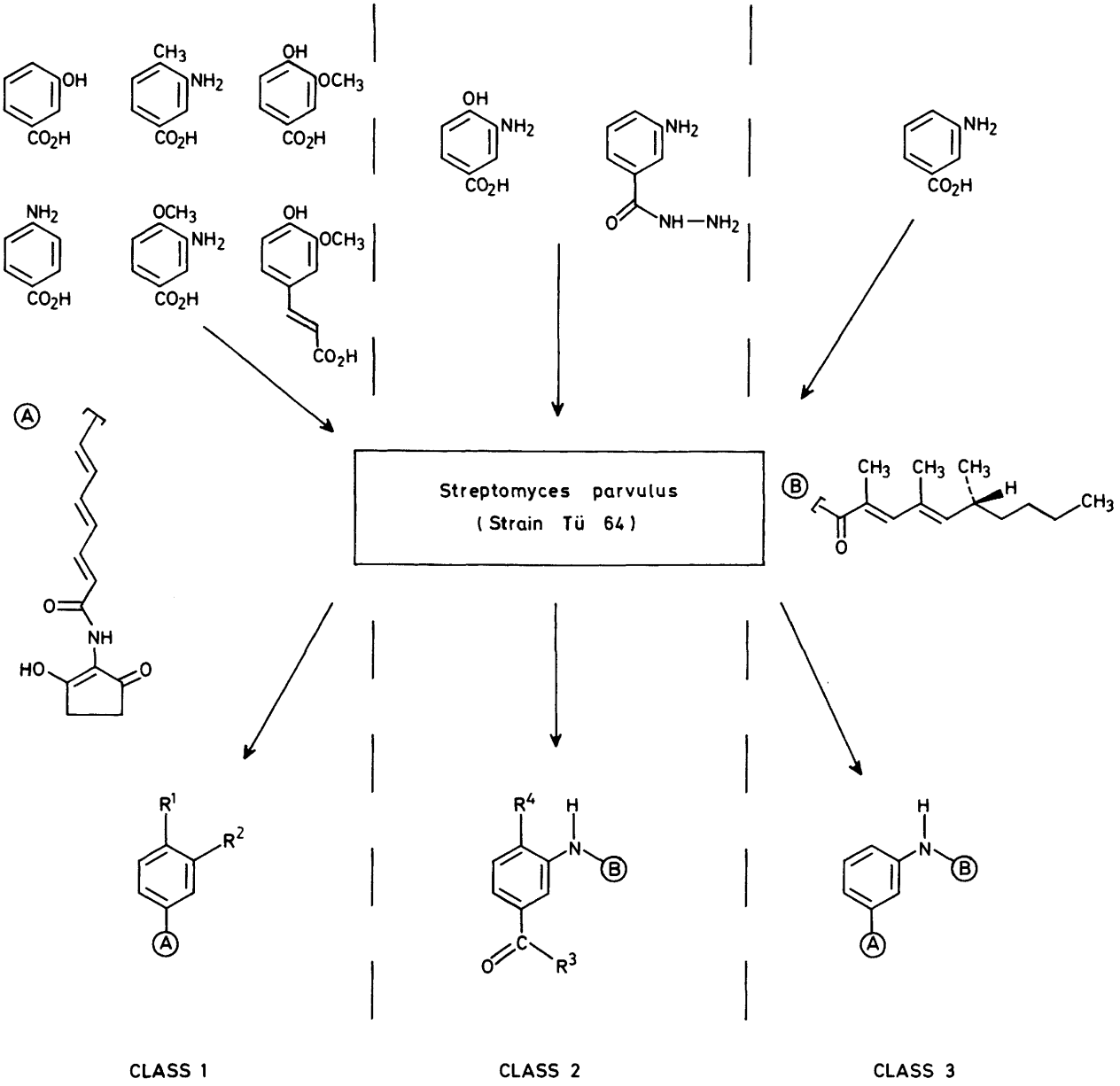
Precursor (55 mmol l^{-1})	Manumycin (mg l^{-1})	Prodigiosins[a]
3-Acetaminobenzoic acid		S
3-Amino-4-chlorobenzoic acid		W
3-Aminocyclohexanecarboxylic acid	8.2	S
4-Aminocyclohexanecarboxylic acid		S
4-Amino-2-hydroxybenzoic acid		W
5-Amino-2-nitrobenzoic acid		S
4-Aminophenylacetic acid		S
5-Aminosalicylic acid		S
Methyl 3-aminobenzoate		W
Benzoic acid		VW
3-Fluorobenzoic acid		W
4-Fluorobenzoic acid		W
Gallic acid	10.2	VW
2-Hydroxy-3-methoxybenzoic acid		W
Nicotinic acid	7.0	VW
3-Nitrobenzoic acid		S
4-Nitrobenzoic acid		S
Phenylacetic acid		S
Protocatechuic acid	25.6	VW
Shikimic acid		S
3-Aminoacetophenone		VW
3-Aminobenzaldehyde		W
3-Aminobenzonitrile		VW
3-Aminobenzyl alcohol		W
Phenylene-1,3-diamine		W
Vanillic aldehyde		VW

[a] See Table 1: S = strong, W = weak, VW = very weak.

in nearly complete coalescence and hence corresponding line-broadening. The carbon signal at 115 p.p.m. (C-2″) definitely indicates the existence of the C_5N moiety. In addition, the e.i.-mass spectra of compounds (**2**)—(**7**) showed a common fragmentation pattern (Scheme 1), consisting of an α-fragmentation on both sides of the C-13 amide carbonyl group ($M - 112$, $M - 140$) and a selective splitting within the triene chain between C-9 and C-10 in which the aromatic part of the molecule is stabilized by the uptake of one hydrogen atom ($M + 1 - 179$). The fragment ion at m/z 193 is a direct indicator of the chiral C_{13}-side chain in the manumycin analogues [*e.g.* (**2**), (**8**), and (**9**)].

Because of the complexity of the ^1H n.m.r. spectra we were unable to analyse the stereochemistry of the triene chains with the exception of the double bond at C-11, which is *trans* in all new manumycin analogues. Since 64-*m*ABA (**2**) exhibits an all-*trans* triene chain, as does the parent antibiotic manumycin (**1**),[9] we postulate the same configuration for the new manumycin analogues. The stereochemistry of the diene chains in compounds (**8**) and (**9**) were not examined, but the chemical shifts of the ^1H n.m.r. signals are so similar to those of manumycin that we postulate conformity. Compounds 64-3A4Hy (**8**) and 64-BZH (**9**) showed similar optical rotation values to 64-*m*ABA (**2**) and (*R*)-2,4,6-trimethyldeca-2,4-dienoic acid,[9] the absolute configurations of which had already been established.[11] It is probable that in the manumycin-analogues (**2**), (**8**), and (**9**) the chiral C_{13}-side chain has the same chirality as in the parent antibiotic manumycin (**1**).

Although the natural *m*-C_7N unit is expected to possess an amino functionality in *meta*-disposition to a free carboxy group,



Scheme 2. Survey on the fed and incorporated aromatic precursors and the three classes of manumycin analogues

it is noteworthy that precursors with a totally different substitution pattern on the aromatic ring are used by *Streptomyces parvulus* to produce manumycin analogues. Scheme 2 illustrates the biosynthetic variability of our strain. Based on the structures of the known manumycin analogues we are able to divide them into three classes:

Class 1: The precursor is enlarged by the triene chain including the C_5N moiety [64-*p*ABA (**3**),[1] 64-HBA (**4**), 64-3A4M (**5**), 64-3A4MO (**6**), and 64-VAN (**7**)].

Class 2: The precursor is linked to the chiral C_{13}-side chain only [64-3A4Hy (**8**) and 64-BZH (**9**)].

Class 3: The precursor is connected to both structural elements, thus exhibiting the entire carbon skeleton of the parent antibiotic manumycin (**1**) [64-*m*ABA (**2**)[1]].

Use of 3-amino-4-hydroxybenzoic acid (3A4Hy) as *m*-C_7N starter unit resulted in 64-3A4Hy (**8**), in contrast to 5-aminosalycilic acid which only blocked the manumycin biosyntheses without the production of a new metabolite. In addition, [7-^{13}C]-3-amino-5-hydroxybenzoic acid (AHBA) fed in physiological amounts was not incorporated into manumycin (**1**).[4] These results are in accordance with the prediction[1,4] that hydroxylated 3-aminobenzoic acids are not intermediates in the manumycin biosynthesis.

Discussion

Based on the eight known manumycin analogues (**2**)—(**9**) formed by application of the precursor-directed biosynthetic method it seems to be possible to differentiate between two enzymes or enzyme systems which are essential for the incorporation of the precursor as central starter units. One of them, probably an amidase, is responsible for the connection of the chiral C_{13}-carboxylic acid to the amino group, and the other activates the carboxy group (CoA-transferase) for the subsequent chain extension process *via* the polyketide pathway.[12] The latter may be part of a multienzyme complex. Because of

the appearance of three different classes of metabolites one may conclude that the amidase and the CoA-transferase act in non-coupled processes (Scheme 2). It seems likely that the amidase is more sensitive to the structure of the artificial precursor than the transferase activating the aromatic carboxy group. It is not, as yet, clear why elongation of 3-amino-4-hydroxybenzoic acid at the carboxy group fails. In the case of 3-hydroxybenzoic acid, the parent antibiotic manumycin (**1**) (33 mg/l) was isolated together with (**4**) (7.5 mg/l). Thus, 3-hydroxybenzoic acid does not compete very successfully with the natural *m*-C_7N precursor. This contrasts with the other feeding experiments (Table 1) in which the manumycin biosynthesis was suppressed totally.

In none of our fermentations to produce manumycin or manumycin analogues did we observe any compounds exhibiting variations in the length of the triene chain. It therefore seems likely that the polyketide synthetase involved in the chain formation is highly specific in its action. This was confirmed by feeding experiments with vanillic acid (4-hydroxy-3-methoxybenzoic acid, VAN) and ferulic acid (4-hydroxy-3-methoxycinnamic acid, FER) (Table 1 and Scheme 2) in which it was found that both starter molecules initiate the formation of the same product, 64-VAN (**7**). With the exception of C-11/C-12 the configuration of the double bonds has not been determined because of signal overlapping in the ^1H n.m.r. spectrum. The termination of the triene chain elongation seems to be under the strict control either of the polyketide synthetase itself or of a second amidase which is responsible for the connection of the activated triene carboxylic acid to the C_5N unit. This seems to be valid for all other manumycin-group antibiotics with the exception of colabomycin,[13] in which a tetraene chain was formed by the producing micro-organism.

Besides the aromatic compounds, which led to the manumycin analogues described above (Scheme 2), we carried out further feeding experiments using different acids, amines, and amino acids (Table 2). Most of these precursors suppressed the manumycin biosynthesis totally, but did not result in any new metabolite. At this state of our investigation we cannot decide whether the negative results are due to the specificity of the enzymes involved (amidase, CoA-transferase) or to other effects, *e.g.* transport into the cells, inhibition of the secondary metabolism, toxic side effects, and wrong timing and/or concentrations during the feeding experiments.

All in all the production of the manumycin analogues described here does highlight the variability of the precursor-directed biosynthetic method developed for the manumycin producer. The replacement of a central starter unit in other antibiotics (not only natural products containing a *m*-C_7N unit) by increasing concentrations of suitable precursors may lead to interesting derivatives produced by micro-organisms and may reinforce our method as an alternative to chemical derivatization, especially in the case of high-cost drugs.

Experimental

General procedure was as described in ref. 1.

Fermentation and Feeding Experiments.—The fermentation conditions for *Streptomyces parvulus* (strain Tü 64) have been described in detail.[9] Feeding experiments with various precursors (see Tables 1 and 2) were carried out using 100 ml of culture medium (2% mannitol; 2% degreased soybean meal) in 1000 ml Erlenmeyer flasks which were shaken for 72 h; 36—40 h after inoculation the precursor, dissolved in a small amount of sterile water and adjusted to pH 7.0 with 2M NaOH, was added under sterile conditions. The cultures were harvested as described for manumycin[9] and the crude products were screened by t.l.c. in various solvent systems (see Tables 1 and 2).

N-(2-*Hydroxy*-5-*oxocyclopent*-1-*enyl*)-7-(3-*hydroxyphenyl*)-*hepta*-2,4,6-*trienamide* (64-HBA), (**4**).—The dark red crude product was chromatographed on a silica gel column [35 × 2.5 cm; $CHCl_3$–MeOH(9:1 v/v)], and the resulting brown product was stirred with ethanol (20 ml) at room temperature and filtered. The filter cake was rechromatographed on a silica gel column [20 × 1.5 cm; $CHCl_3$–MeOH (4:1 v/v)] to give the pure *amide* (**4**) (7.5 mg l^{-1}), m.p. 263 °C; R_F 0.76 [$CHCl_3$–MeOH (4:1 v/v)]; $\nu_{max.}$(KBr) 3 420, 3 260, 1 610, 1 580sh, 1 545sh, and 1 005 cm^{-1}; $\lambda_{max.}$(MeOH) 357 (ε 39 400), 262 (24 600), and 202 nm (34 000); $\lambda_{max.}$(MeOH–HCl) 363 (ε 42 300), 263 (19 700), and 202 nm (32 300); $\lambda_{max.}$(MeOH–NaOH) 343 (ε 57 300), 361 (53 800), and 210 nm (144 600); δ_C[$(CD_3)_2SO$] 166.0 (s, C-13), 157.5 (s, C-2), 142.3 (d, C-11), 141.0 (d, C-9), 137.7 (s, C-4), 136.4 (d, C-7), 121.5 (d, C-12), 117.8 (d, C-5), 115.6 (d, C-3), 114.8 (s, C-2″), 113.1 (d, C-1), and 28.8 (br t, C-4″ and C-5″) [the signals at 130.4 (d), 129.6 (d) and 128.2 (d) could not be assigned definitely to C-6, C-8, and C-10; the signals fr C-1″ and C-3″ were not observed at 50.3 MHz]; δ_H[$(CD_3)_2SO$] 2.06 (br s, 4″-H_2 and 5″-H_2), 6.48—7.23 (9 H, m), 7.30 (dd, *J* 15 and 12 Hz, 11-H), 9.41 (br s, NH or OH), 9.91 (br s, NH or OH), and 13.80 (br s, OH); *m/z* 311.1157 (26%, M^+; $C_{18}H_{17}NO_4$) 199 (42), 198 (10), 171 (43), 153 (41), and 132 (100).

7-(3-*Amino*-4-*methylphenyl*)-N-(2-*hydroxy*-5-*oxocyclopent*-1-*enyl*)*hepta*-2,4,6-*trienamide* (64-3A4M), (**5**).—The dark red crude product was chromatographed twice on a silica gel column [35 × 2.5 cm; $CHCl_3$–MeOH (9:1 v/v)] and was further purified on a Sephadex LH-20 column (50 × 2.5 cm; $CHCl_3$) to yield the yellow amorphous *product* (**5**) (12.1 mg l^{-1}), m.p. 289 °C (decomp.); $\nu_{max.}$(KBr) 3 480, 3 260, 1 594, and 1 006 cm^{-1}; $\lambda_{max.}$(MeOH) 355 (ε 21 400) and 260 (11 000); $\lambda_{max.}$(MeOH–HCl) 353 (ε 43 900) and 256 (10 000); $\lambda_{max.}$(MeOH–NaOH) 348 (ε 34 700) and 260 (20 900); δ_C[$(CD_3)_2SO$] 166.1 (s, C-13), 146.6 (s, C-2), 142.6 (d, C-11), 141.5 (d, C-9), 137.5 (d, C-7), 134.7 (s, C-4), 130.2 (d, C-10), 129.4 (d, C-6), 126.6 (d, C-8), 122.2 (s, C-1), 120.9 (d, C-12), 115.2 (d, C-5), 114.9 (s, C-2″), 112.0 (d, C-3), 28.9 (br t, C-4″ and C-5″), and 17.2 (q, CH_3) (the signals for C-1″ and C-3″ were not observed at 50.3 MHz); δ_H[$(CD_3)_2SO$] 1.26 (s, Me), 2.07 (br s, 4″-H_2), 2.49 (br s, 5″-H_2), 3.30 (br s, NH overlapped by HOD), 6.46—7.02 (8 H, m), 7.33 (dd, *J* 15 and 11.5 Hz, 11-H), and 9.90 (br s, OH); *m/z* 324.1474 (61%, M^+; $C_{19}H_{20}N_2O_3$), 212 (20), 197 (5), 184 (34), 169 (20), and 146 (51).

7-(3-*Amino*-4-*methoxyphenyl*)-N-(2-*hydroxy*-5-*oxocyclopent*-1-*enyl*)*hepta*-2,4,6-*trienamide* (64-3A4MO), (**6**).—The crude product was chromatographed on a silica gel column [35 × 2.5 cm; $CHCl_3$–MeOH (9:1 v/v)] and was further purified on a Sephadex LH-20 column (90 × 2.5 cm; MeOH) and on preparative t.l.c. [silica gel on glass; $CHCl_3$–MeOH (9:1 v/v)] to yield the yellow amorphous *product* (**6**) (28.4 mg l^{-1}), m.p. 258 °C; $\nu_{max.}$ 3 450, 3 360, 3 230, 1 688sh, 1 608, 1 570, and 1 002 cm^{-1}; $\lambda_{max.}$(MeOH) 366 (ε 38 300) and 260 (26 200); $\lambda_{max.}$(MeOH–HCl 365 (ε 49 900) and 261 (13 000); $\lambda_{max.}$(MeOH–NaOH) 365 (ε 38 600), 260 (26 000), and 206 (69 200); δ_C[$(CD_3)_2SO$] 166.2 (s, C-13), 147.2 (s, C-1), 142.7 (d, C-11), 141.8 (d, C-9), 137.7 (s, C-2), 137.4 (d, C-7), 129.3 (s, C-4), 128.7 (d, C-10), 125.4 (d, C-8), 120.4 (d, C-12), 116.4 (s, C-2″), 114.9 (d, C-5), 111.2 (d, C-3), 110.5 (d, C-6), 55.3 (q, OCH_3), and 28.7 (br t, C-4″ and C-5″) (the signals for C-1″ and C-3″ are not observable at 50.3 MHz); δ_H[$(CD_3)_2SO$] 3.30 (6 H, br m, 4″-H_2, 5″-H_2, NH), 3.81 (s, OCH_3), 6.42—7.04 (9 H, m), 7.33 (dd, *J* 15 and 11.5 Hz, 11-H), 9.92 (br s, OH), and 13.82 (br s, OH); *m/z* 340.1424 (18%, M^+; $C_{19}H_{20}N_2O_4$), 292 (7), 243 (10), 228 (12), 200 (29), 162 (100), and 97 (22).

7-(4-*Hydroxy*-3-*methoxyphenyl*)-N-(2-*hydroxy*-5-*oxocyclopent*-1-*enyl*)*hepta*-2,4,6-*trienamide* (64-VAN), (**7**).—The dark

red crude product was chromatographed twice on a silica gel column [35 × 2.5 cm; $CHCl_3$–MeOH (9:1 v/v)] and was further purified on a Sephadex LH-20 column (90 × 2.5 cm, $CHCl_3$) to yield 23 mg l^{-1} (20 mg l^{-1} ferulic acid feeding experiment) of yellow amorphous *amide* (**7**), m.p. 214 °C; $\nu_{max.}$ 3 440, 2 930, 1 618sh, 1 600sh, 1 588, and 1 003 cm^{-1}; $\lambda_{max.}$(MeOH) 364 (ε 26 700) and 260 (16 900); $\lambda_{max.}$(MeOH–HCl) 384 (ε 28 000) and 268 (10 100); $\lambda_{max.}$(MeOH–NaOH) 417 (ε 23 100), 260 (17 100), and 210 (13 300); $\delta_C[(CD_3)_2SO]$ 188.9 (br s, C-1″), 165.1 (s, C-13), 165.0 (br s, C-3″), 147.8 (s, C-1 or C-2), 147.5 (s, C-1 or C-2), 141.0 (d, C-11), 140.3 (d, C-9), 136.2 (d, C-7), 129.1 (d, C-8 or C-10), 128.2 (s, C-4), 127.7 (d, C-8 or C-10), 125.7 (d, C-5), 122.4 (d, C-12), 120.7 (d, C-6), 115.6 (s, C-2″), 110.0 (d, C-3), 55.6 (t, OCH_3), 29.7 (t, C-4″ or C-5″), and 28.8 (t, C-4″ or C-5″); $\delta_H[(CD_3)_2SO]$ 2.08 (br s, 4″-H_2), 2.32 (br s, 5″-H_2), 3.50 (br s, OH), 2.81 (s, OMe), 6.38—7.03 (7 H, m), 7.12 (s, 3-H), 7.23 (dd, *J* 15 and 12 Hz, 11-H), 8.82 (br s, NH or OH), 9.61 (br s, NH or OH), and 13.78 (br s, OH); *m/z* 341.1263 (3.6%, M^+; $C_{19}H_{19}NO_5$), 229 (24), 201 (10), 197 (13), 169 (66), 163 (100), and 131 (67).

4-*Hydroxy*-3-(2,4,6-*trimethyldeca*-2,4-*dienylcarbonylamino*)-*benzoic Acid* (64-3A4Hy), (**8**).—The crude product was chromatographed on a silica gel column [35 × 2.5 cm; $CHCl_3$–MeOH (9:1 v/v)] and was further purified on a Sephadex LH-20 column (90 × 2.5 cm, MeOH) to yield (**8**) as a yellow *oil* (13.3 mg l^{-1}), $[\alpha]_D^{22}$ −15.2° (*c* 0.60 in $CHCl_3$); $\nu_{max.}$(KBr) 3 440, 2 920, 2 860, 1 650sh, 1 629, and 1 596sh cm^{-1}; $\lambda_{max.}$(MeOH) 407 (ε 18 500), 270 (26 200), and 212 (120 100); $\lambda_{max.}$(MeOH–HCl) 407 (ε 18 200), 270 (25 700), and 212 (119 500); $\lambda_{max.}$(MeOH–NaOH) 408 (ε 17 000), 269 (25 200), and 214 (127 200); $\delta_H(CDCl_3)$ 0.88 (t, *J* 6.5 Hz, 10′-H_3), 0.98 (d, *J* 6.5 Hz, 13′-H_3), 1.16—1.40 (br m, 7′-H_2, 8′-H_2, and 9′-H_2), 1.86 (d, *J* 1.6 Hz, 12′-H_3), 2.14 (d, *J* 1.6 Hz, 11′-H_3), 2.36—2.60 (br m, 6′-H), 5.44 (d, *J* 10 Hz, 5′-H), 6.53 (br s, NH or OH), 6.98 (s, 3′-H), 7.51 (d, *J* 8.8 Hz, 6-H), 8.14 (dd, *J* 8.5 and 2 Hz, 5-H), 8.27 (d, *J* 2 Hz, 3-H), 8.52 (s, NH or OH), and 9.07 (br s, COOH); *m/z* 346 (4.3%, MH^+), 193 (9.5), and 153 (8.4).

3-(2,4,6-*Trimethyldeca*-2,4-*dienylcarbonylamino*)*benzo-hydrazide* (64-BZH), (**9**).—The dark red crude product was chromatographed on a silica gel column [20 × 2.5 cm; $CHCl_3$–MeOH (9:1 v/v)] and was further purified on a Sephadex LH-20 column (90 × 2.5 cm; MeOH) to yield pure (**9**) as an *oil* (23.0 mg l^{-1}), $[\alpha]_D^{22}$ −42° (*c* 0.95 in acetone); $\nu_{max.}$(KBr) 3 420, 3 260, 2 960, 2 930, 1 660, 1 630, 1 610, and 1 590 cm^{-1}; $\lambda_{max.}$(MeOH) 254 (ε 20 800) and 223 (20 100); $\lambda_{max.}$(MeOH–HCl) 262 (ε 16 400) and 222 (14 300); $\lambda_{max.}$(MeOH–NaOH) 313 (ε 8 700), 254 (16 700), and 221 (18 600); $\delta_C(CDCl_3)$ 166.0 (s, C-1′), 163.5 (s, C-7), 146.9 (s, C-2), 143.0 (d, C-5′), 140.7 (d, C-3′), 132.5 (s, C-4), 129.9 (s, C-2′ or C-4′), 129.7 (s, C-2′ or C-4′), 125.1 (d, C-6), 118.7 (d, C-1), 116.7 (d, C-5), 113.6 (d, C-3), 37.0 (t, C-7′), 32.9 (d, C-6′), 29.8 (t, C-8′), 22.8 (t, C-9′), 20.8 (q, C-13′), 16.5 (q, C-12′), 14.1 (q, C-10′), and 13.7 (l, C-11′); $\delta_H[(CD_3)_2CO]$ 0.88 (t, *J* 6 Hz, 10′-H_3), 0.97 (d, *J* 6.5 Hz, 13′-H_3), 1.25 (br m, 7′-H_2, 8′-H_2, and 9′-H_2), 1.82 (d, *J* 1.5 Hz, 12′-H_3), 2.07 (d, *J* 1.5 Hz, 11′-H_3), 2.47 (br s, 6′-H), 3.87 (br s, NH), 5.38 (d, *J* 9 Hz, 5′-H), 6.86 (m, 6-H), 6.96 (s, 3-H), and 7.16—7.27 (m, 1-H, 5-H, and 3′-H); *m/z* 343.2260 (28%, M^+; $C_{20}H_{29}N_3O_2$), and 258 (7), 218 (5), 193 (68), 151 (4), and 120 (100%).

Acknowledgements

We are grateful to Professor H. Zähner, Institut für Biologie der Universität Tübingen for providing us with *Streptomyces parvulus* (strain Tü 64) and to M. Lackner and H. Müller for excellent technical assistance. This work was supported by the Fonds der Chemischen Industrie.

References

1 Part 1, R. Thiericke and A. Zeeck, *J. Chem. Soc., Perkin 1*, 1988, 2123.
2 H. G. Floss, in 'Recent Advances in Phytochemistry,' ed. E. E. Conn, Plenum Press, New York, 1986, **20**, p. 13.
3 H. G. Floss, P. J. Keller, and J. M. Beale, *J. Nat. Prod.*, 1986, **49**, 957.
4 J. M. Beale, R. Harrold, H. G. Floss, R. Thiericke, A. Zeeck, A. Nakagawa, and S. Omura, *J. Am. Chem. Soc.*, 1988, **110**, 4435.
5 O. Ghisalba and J. Nüesch, *J. Antibiotics*, 1981, **34**, 64.
6 K. L. Rinehart, Jr., M. Potgieter, D. L. Delaware, and H. Seto, *J. Am. Chem. Soc.*, 1981, **103**, 2099.
7 U. Degwert, R. van Hülst, H. Pape, R. Herrold, J. M. Beale, P. J. Keller, J. P. Lee, and H. G. Floss, *J. Antibiotics*, 1987, **40**, 855.
8 Y. Sato and S. J. Goule, *J. Am. Chem. Soc.*, 1986, **108**, 4625.
9 A. Zeeck, K. Schröder, K. Frobel, R. Grote, and R. Thiericke, *J. Antibiotics*, 1987, **40**, 1530.
10 A. Zeeck, K. Frobel, C. Heusel, K. Schröder, and R. Thiericke, *J. Antibiotics*, 1987, **40**, 1541.
11 R. Thiericke, M. Stellwaag, A. Zeeck, and G. Snatzke, *J. Antibiotics*, 1987, **40**, 1549.
12 R. Thiericke and A. Zeeck, *J. Antibiotics*, 1988, **41**, 694.
13 R. Grote, A. Zeeck, H. Drautz, and H. Zähner, *J. Antibiotics*, 1988, **41**, 1178.

Received 6th April 1988; *Paper* 8/01352G