3-Amino-5-hydroxybenzoic Acid in Antibiotic Biosynthesis. XI.†

Biological Origins and Semisynthesis of Thionaphthomycins, and the Structures of Naphthomycins I and J

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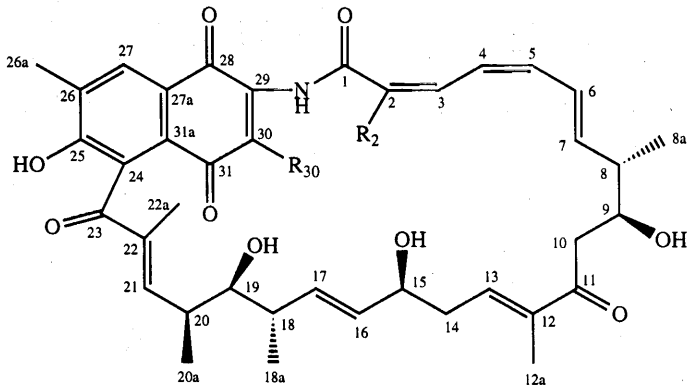
Fermentations of *Streptomyces* sp. E/784 produce low levels of the novel C-30 alkylthio-substituted ansamycin antibiotics naphthomycins J (**9**) and I (**10**), in addition to the more abundant C-30 hydroxylated analogues actamycin (**1**) and naphthomycin D (**2**) and C-30 chlorinated analogues naphthomycins H (**3**) and A (**4**). The addition of *N*-acetyl-L-cysteine to the fermentation medium substantially increases the production of the thionaphthomycins J and I at the expense of their chloro analogues H and A. Other thiols and thiol progenitors are similarly utilised, including *N*-acetyl-L-cysteine methyl ester which affords the known naphthomycin F (**8**) and its novel 2-demethyl homologue (**7**). The formation of thioansamycins from chloroansamycins and thiols *in vivo* is probably non-enzymic since similar conversions can be effected *in vitro*.

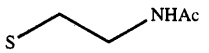
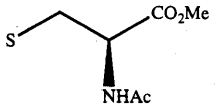
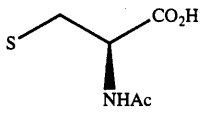
Native antibiotics of the ansamycin group[1~3] display a range of potent biological activities, and the semi-synthetic derivative rifampicin is the antibiotic of choice in the treatment of human *Mycobacterium tuberculosis* infections. Members of the group are characterised by the presence of a mono- or bicyclic aromatic or quinonoid nucleus across which a polyketide chain is linked to form a macrocyclic lactam. The naphthalenoid ansamycins, exemplified by actamycin (**1**) and the naphthomycins (**2**~**4** and **8**), carry a variety of nuclear substituents ranging from hydrogen to methyl, hydroxyl, methoxyl, chloro, and alkylthio at the C-30 position adjacent to the lactam function. This site corresponds to the 4-position of the 3-amino-5-hydroxybenzoic acid (AHB) precursor which initiates formation of the polyketide carbon skeleton of these antibiotics.[4~6] Appropriately substituted analogues of AHB are not incorporated into the final antibiotics, however, and the C-30 functionality is believed to be introduced late in the biosynthetic process.[7,8] We describe here fermentation and semi-synthetic studies relating to the stage and mode of biological introduction of the alkylthio substituents into sulfur-containing members of the naphthomycin group.

Results and Discussion

During an investigation into the early stages of the biosynthesis of naphthalenoid ansamycins (unpublished work), the deuterated *N*-acetylcysteamine thioesters (**11**) and (**12**) (as racemates) were prepared and introduced to separate fermentations of *Streptomyces* sp. E/784, a bacterium known to produce actamycin (**1**)[9] and naphthomycins D (**2**),[10] H (**3**),[11] and A (**4**).[10,12,13] Examination of the metabolites formed after fermentation for 100 hours showed normal production of the C-30 hydroxylated compounds (**1**) and (**2**), but almost undetectable levels of the analogous C-30 chlorinated co-metabolites naphthomycins H (**3**) and A (**4**). The actamycin (**1**) formed in each case showed no significant deuterium incorporation by ^2H NMR spectroscopy. Both fermentations, however, produced two novel orange naphthomycins (**5**) and (**6**), with physical properties, ^{13}C and ^1H NMR data as recorded in Tables 1, 2 and 3. Electrospray mass spectrometry indicated the molecular formulae $C_{43}H_{52}N_2O_{10}S$ and $C_{44}H_{54}N_2O_{10}S$, corresponding to the replacement of the chlorine substituent in naphthomycins H (**3**) and A (**4**) respectively with a

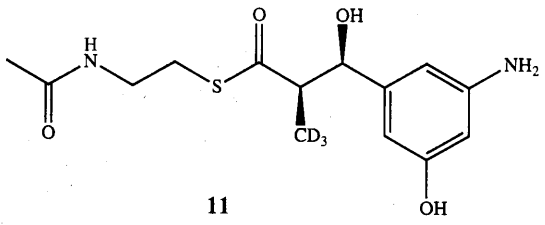
† Part X. ANDERSON, M. G.; D. MONYPENNY, R. W. RICKARDS & J. M. ROTHSCHILD: Biosynthetic origins of the oxygen atoms in the ansamycin antibiotics rifamycin B, O, and S. J. Chem. Soc., Chem. Commun. 311~313, 1989.



	R_{30}	R_2	
1	OH	H	Actamycin
2	OH	Me	Naphthomycin D
3	Cl	H	Naphthomycin H
4	Cl	Me	Naphthomycin A
5		H	
6		Me	
7		H	2-Demethylnaphthomycin F
8		Me	Naphthomycin F
9		H	Naphthomycin J
10		Me	Naphthomycin I

C_4H_8NOS moiety. This moiety was in each case assigned the 2-acetylaminoethanethio structure by ^1H, ^{13}C and correlation NMR spectroscopy. Addition-elimination reactions of thiols with chloronaphthoquinones are well known,[14,15] and this process had presumably occurred under the fermentation conditions between *N*-acetyl cysteamine, formed on hydrolysis of the added thioesters (**11** and **12**), and the chlorinated naphthomycins (**3**) and (**4**).

The literature reports previous isolations from bacterial fermentations of a range of natural thioansamycins,[††] all of which carry the sulfur substituent at the same quinonoid position. Of particular interest in the present context are naphthomycin F (**8**), and naphthomycin G which has been assigned the structure (**10**).[10] In structural terms, these are the carbomethoxylated and carboxylated analogues, respectively, of the novel thioansamycin (**6**), and could reflect the incorporation of L-cysteine units or their derivatives into the chlorinated naphthomycin A (**4**). In agreement, supplementation of



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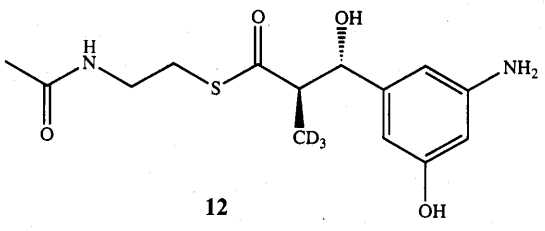

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a fermentation of *Streptomyces* sp. E/784 with the methyl ester of *N*-acetyl-L-cysteine again dramatically reduced the levels of naphthomycins H (**3**) and A (**4**), while naphthomycin F (**8**) and the previously unreported

†† Apart from those mentioned in this paper, these include ansathiazin, awamycin, CP 50833, naphthoquinomycin B, 3-(methylthio)rifamycin, and TAN 528A.[16]

Table 1. Physico-chemical properties of naphthomycins (5~10).

	5	6	7	8	9	10
Formula	$C_{43}H_{52}N_2O_{10}S$	$C_{44}H_{54}N_2O_{10}S$	$C_{45}H_{54}N_2O_{12}S$	$C_{46}H_{56}N_2O_{12}S$	$C_{44}H_{52}N_2O_{12}S$	$C_{45}H_{54}N_2O_{12}S$
Appearance	Orange precipitate	Orange precipitate	Orange solid	Orange solid	Orange precipitate	Orange solid, Orange prisms[b]
MP °C	124~128	132~136	114~118	142~144	147~151	167~171, 188 (dec.)[b]
$[\alpha]_D^{22}$	+432° ($CHCl_3$)	+469° ($CHCl_3$)	+352° ($CHCl_3$)	+444° ($CHCl_3$), +334.4° ($CHCl_3$)[b]	+398° ($CHCl_3$)	+228° (MeOH), +254° (MeOH)[b]
FAB-MS (*m/z*)				883 $(M+Na)^+$		869 $(M+Na)^+$
ES-MS (*m/z*)	789 $(M+H)^+$, 806 $(M+NH_4)^+$, 811 $(M+Na)^+$	803 $(M+H)^+$, 820 $(M+NH_4)^+$, 825 $(M+Na)^+$	847 $(M+H)^+$, 864 $(M+NH_4)^+$, 869 $(M+Na)^+$	861 $(M+H)^+$, 878 $(M+NH_4)^+$, 883 $(M+Na)^+$	833 $(M+H)^+$, 850 $(M+NH_4)^+$, 855 $(M+Na)^+$	847 $(M+H)^+$, 864 $(M+NH_4)^+$, 869 $(M+Na)^+$
IR ν_{max} ($CHCl_3$) cm^{-1}	1710, 1655	1720, 1655	1740, 1655	1740, 1655 1745, 1660 (KBr)[b]	1720, 1655	1725, 1650 1728, 1665 (KBr)[b]
UV λ_{max} (MeOH) nm	233 (4.44), 289 (4.27), 308 (4.34)	235 (4.57), 283 (4.51), 361 (3.80)	237 (4.35), 283 (4.27), 305 (4.20)	236 (4.56), 284 (4.54), 347 (3.82), 236 (4.59)[b], 285 (4.50)[b], 320 (4.34)[b]	234 (4.39), 287 (4.29), 306 (4.31)	234 (4.59), 283 (4.56), 351 (3.84), 230 (4.64)[b], 283 (4.36)[b], 360 (3.76)[b]
HR ES-MS amu	Found 789.342 $C_{43}H_{53}N_2O_{10}S$ requires 789.342	Found 803.356 $C_{44}H_{55}N_2O_{10}S$ requires 803.358	Found[a] 869.329 $C_{45}H_{54}N_2NaO_{12}S$ requires 869.330	Found 878.386 $C_{46}H_{60}N_3O_{12}S$ requires 878.390	Found 833.333 $C_{44}H_{53}N_2O_{12}S$ requires 833.332	Found 869.325 $C_{45}H_{54}N_2NaO_{12}S$ requires 869.330

[a] FAB-MS amu. [b] See reference 10).

homologue 2-demethylnaphthomycin F (**7**) were now produced. The structures of these metabolites followed from their molecular formulae $C_{46}H_{56}N_2O_{12}S$ and $C_{45}H_{54}N_2O_{12}S$, and from their ^{13}C and ^1H NMR spectral data (Tables 2 and 3) in conjunction with the known structure and stereochemistry of the metabolites[10~13] which they have replaced. Confirmation of these assignments followed from the identity of the spectroscopic data of the isolated naphthomycin F with that previously reported.[10]

Supplementation of the Streptomycete fermentation in similar fashion with *N*-acetyl-L-cysteine produced, as expected, two homologous naphthomycins with molecular formulae $C_{45}H_{54}N_2O_{12}S$ and $C_{44}H_{52}N_2O_{12}S$, again at the expense of naphthomycins H (**3**) and A (**4**). In view of the above results, the former product was expected to be the known naphthomycin G, with the assigned structure (**10**),[10] the latter its novel 2-demethyl homologue (**9**). That these structures are correct for the present compounds follows from the similarity of their spectral data with that of naphthomycin F (**8**) and its 2-demethyl homologue (**7**) (Tables 1, 2 and 3). Furthermore, these structures are in agreement with those of the presumed precursors, the chlorinated naphthomycins H (**4**) and A (**3**) which are the normal fermentation products. Surprisingly, however, the ^{13}C and ^1H NMR spectral data of the higher C_{45} homologue differed from those reported for naphthomycin G.[10] We conclude that the stereochemistry reported for naphthomycin G may be in error, but have been unable to obtain an authentic sample.

A detailed retrospective examination of the extracted metabolites of *Streptomyces* sp. E/784 after normal unsupplemented fermentation for 72 hours revealed, in addition to the previously observed actamycin (**1**) and naphthomycins D (**2**), H (**3**) and A (**4**), low natural production of the novel naphthomycin (**10**) and its 2-demethyl homologue (**9**). Since they are thus true natural products, we have named them naphthomycins I and J, respectively. Naphthomycin J (**9**) is an ansa-chain stereoisomer of the known thioansamycin diastovaricin II.[17]

Confirmation of the structures of the thionaphthomycins produced in the fermentations, and support for the mode of introduction of the alkylthio substituents, was obtained by *in vitro* semisynthetic studies. Treatment of naphthomycin A (**4**) with *N*-acetyl cysteamine or *N*-acetyl-L-cysteine in water at pH 8, to mimic the fermentation conditions, afforded the novel naphthomycins (**6**) and I (**10**) in good yields. Similar addition-elimination reactions of naphthomycin A with simple alkyl and aryl thiols have been reported by OKABE and co-workers.[18]

In contrast to the definitive results obtained with the *N*-acetyl derivatives of L-cysteine and its methyl ester, the addition of the free amino acid itself to the Strepto-

Table 2. ^{13}C NMR data for naphthomycins (**5**~**10**)[a].

	5	**6**	**7**	**8**	**9**	**10**
Ketone C=O	203.8	203.9	203.8	203.9	203.8	202.0
	201.9	202.1	201.8	202.2	201.7	199.3
Quinone C=O	182.0	182.3	181.2	181.5	181.3	182.1
	178.4	178.4	178.3	178.5	178.6	178.7
C-1 Amide	165.6	169.4	166.0	169.5[b]	166.2	169.6
C=C	160.4	160.7	160.5	160.9	160.0	159.1
	147.5	147.4	147.4	147.2	147.8	147.5
	142.7	143.0	142.7	143.0	142.5	141.8
	141.7	140.0	141.7	140.5	141.6	140.7
	140.3	140.0	141.2	140.2	141.4	139.2
	138.1	137.8	138.0	137.9	138.1	137.7
	137.4	137.4	137.4	137.4	137.4	137.5
	136.5	136.3	136.6	136.4	136.2	137.1
	136.3	135.7	136.2	135.4	136.2	136.7
	135.7	134.8	135.7	134.0	135.5	134.9
	135.5	134.2	135.2	133.4	135.0	133.9
	133.9	133.2	133.7	133.3	134.1	132.7
	133.5	132.2	133.1	132.4	133.3	132.6
	133.1	131.2	132.4	131.0	132.8	131.4
	131.0	131.0	130.9	131.0	130.8	130.3
	126.5	129.6	126.5	129.9	126.6	128.7
	123.8	126.8	123.7	126.8	124.0	127.2
	121.9	123.6	121.7	123.6	122.1	124.9
	120.9	122.0	121.0	121.8	121.3	123.5
	120.3	120.4	120.4	120.4	120.9	123.2
C–O	76.6	77.0	76.2	76.9	76.4	77.1
	73.4	73.1	73.5	73.3	73.6	72.6
	71.6	71.8	71.6	71.7	71.8	72.1
CH, CH_2	45.1	45.0	45.1	45.0	45.0	46.0
	41.8	41.7	41.8	41.8	41.8	41.9
	40.4	40.6	40.4	40.3	40.4	41.8
	36.5	36.3	36.5	36.3[b]	36.4	36.5
	33.8	33.7	33.6	33.7	33.8	34.2
CH_3	—	20.6	—	20.5	—	20.1
	17.5	17.3	17.5	17.3	17.5	17.4
	16.5	16.5	16.4	16.5	16.5	16.2
	16.3	16.3	16.2	16.3	16.3	16.0
	12.4	12.5	12.3	12.5	12.3	11.7
	11.3	11.1	11.2	11.1	11.3	10.8
	10.9	10.6	10.8	10.6	10.9	10.7
C-30 substituent						
CONH	170.2	170.2	170.8	170.8	171.0[b]	170.2[b]
CO_2	—	—	169.7	169.6[b]	172.4[b]	171.3[b]
NCH	—	—	52.4[b]	52.5[b]	52.7	53.1
CO_2CH_3	—	—	52.1[b]	52.1[b]	—	—
NCH_2	39.2	39.5	—	—	—	—
SCH_2	34.0	34.4	35.8	36.2[b]	35.6	35.6
$COCH_3$	23.1	23.0	22.9	23.1	22.8	22.2

[a] Chemical shifts (δ) at 75.5 MHz for solutions in $CDCl_3$, except for naphthomycin I (**10**) which is in D_6-Me_2CO.
[b] Assignments of adjacent signals may be interchanged.

mycete fermentation resulted in the formation of a large number of metabolites, none of which corresponded to the thionaphthomycins (**7**), (**8**), (**9**) or (**10**). This may indicate that *N*-acetylation of the L-cysteine is necessary before its addition to the chloroquinones, in order to avoid subsequent cyclisation onto the adjacent quinone carbonyl group and further reactions such as occur in the formation of the thiazinone and thiazole rings of the ansamycin antibiotics rifamycins verde, P and Q,[19~21] and the thiazole ring of luciferin.[22]

In view of the ease with which *in vitro* addition-elimination reactions occur, the conversion of chloro-

Table 3. ^{1}H NMR data for naphthomycins (**5**~**10**)[a].

	5	**6**	**7**	**8**	**9**	**10**
25-OH	9.29 (br s)	9.40 (s)	9.33 (br s)	9.45 (br s)	—	9.25 (br s)
29-NH	8.56 (s)	8.46 (s)	8.63 (br s)	8.37 (s)	8.80 (s)	8.68 (s)
27-H	7.89 (s)	7.91 (s)	7.88 (s)	7.93 (s)	7.82 (s)	7.85 (s)
3-H	6.94 (t, 11.4)	6.61 (d, 11.1)	6.95 (t, 11.1)	6.63 (d, 11.4)	6.93 (t, 11.6)	6.59 (d, 11.4)
13-H	6.69 (t, 5.8)	6.76 (t, 5.6)	6.67 (t, 6.7)	6.75 (t, 5.8)	6.66 (br t, 6.4)	6.73 (br t, 6.2)
6-H	6.47	6.47	6.47	6.47	6.47	6.45
	(dd, 11.5, 14.5)	(dd, 11.4, 14.8)	(dd, 11.3, 14.9)	(dd, 11.3, 14.8)	(br t, 13.7)	(dd, 11.3, 14.7)
4-H	6.47 (t, 11.0)	6.28 (t, 11.1)	6.48 (t, 10.7)	6.28 (t, 11.1)	6.51 (t, 11.3)	6.31 (t, 11.0)
5-H	6.27 (t, 11.0)	6.10 (t, 11.1)	6.27 (t, 10.9)	6.13 (t, 11.1)	6.27 (t, 11.2)	6.10 (t, 11.0)
2-H	6.02 (d, 11.4)	—	6.06 (d, 11.3)	—	6.07 (d, 11.9)	—
21-H	5.92	5.94	5.89	5.91	5.87	5.91
	(dd, 1.5, 10.3)	(dd, 1.3, 10.3)	(dd, 1.4, 10.2)	(dd, 1.2, 9.9)	(dd, 1.3, 10.0)	(d, 10.3)
16-H	5.57	5.59	5.55	5.58	5.54 (m[b])	5.58
	(dd, 6.6, 14.8)	(dd, 7.1, 15.0)	(dd, 6.9, 15.1)	(dd, 7.0, 15.8)		(dd, 6.7, 15.1)
17-H	5.49	5.47	5.46	5.47	5.54 (m[b])	5.45[c]
	(dd, 8.6, 15.1)	(dd, 9.1, 14.9)	(dd, 8.8, 14.9)	(dd, 8.9, 15.0)		(dd, 8.8, 14.9)
7-H	5.53 (m)	5.46	5.53	5.48	5.54 (m[b])	5.53[c]
		(dd, 10.1, 14.9)	(dd, 10.2, 14.7)	(dd, 9.9, 15.1)		(dd, 8.7, 15.2)
15-H	4.04 (q, 6.6)	4.02 (q, 6.8)	4.02 (br q, 6.7)	4.00 (m)	4.06 (br q, 6.0)	4.05 (br q, 6.6)
9-H	3.58 (m)	3.54 (m)	3.55 (m)	3.54 (m)	3.58 (m[b])	3.54 (m)
19-H	3.13	3.17 (m[b])	3.11	3.14	3.12	3.12
	(dd, 2.5, 9.9)		(dd, 2.5, 10.1)	(br d, 8.9)	(d, 8.2)	(dd, 2.3, 9.3)
10a-H	3.07	3.17 (m[b])	3.07	3.17	3.02	3.09
	(dd, 3.4, 16.6)		(dd, 3.4, 16.3)	(dd, 2.8, 16.8)	(br d, 15.5)	(dd, 2.6, 16.9)
20-H	2.66 (m)	2.69 (m)	2.66 (m)	2.69 (br d, 8.9)	2.70 (m)	2.68 (m)
10b-H	2.66	2.56	2.64	2.60	2.66 (m)	2.65
	(dd, 5.8, 16.9)	(dd, 6.8, 17.0)	(dd, 5.2, 16.5)	(dd, 6.4, 17.0)		(dd, 6.6, 16.9)
26a-H_3	2.34 (s)	2.36 (s)	2.33 (s)	2.37 (s)	2.32 (s)	2.33 (s)
8-H, 14-H_2	2.27 (3H, m)	2.28 (3H, m)	2.26 (3H, m)	2.28 (3H, m)	2.28 (3H, m)	2.30 (3H, m)
18-H	2.16 (m)	2.16 (m)	2.14 (m)	2.16 (m)	2.14 (m)	2.16 (m)
2a-H_3	—	2.11 (s)	—	2.13 (s)	—	2.10 (s)
22a-H_3	2.00 (s)	2.01 (d, 1.3)	2.00 (d, 1.2)	2.03 (s)	2.00 (s)	2.00 (s)
12a-H_3	1.69 (s)	1.70 (s)	1.68 (s)	1.70 (s)	1.69 (s)	1.69 (s)
8a-H_3	1.18 (d, 6.4)	1.18 (d, 6.5)	1.17 (d, 6.5)	1.19 (d, 6.5)	1.17 (d, 6.5)	1.16 (d, 6.4)
18a-H_3	0.94 (d, 6.6)	0.94 (d, 6.7)	0.92 (d, 6.7)	0.95 (d, 6.6)	0.94 (d, 6.5)	0.94 (d, 6.6)
20a-H_3	0.78 (d, 6.7)	0.80 (d, 6.6)	0.77 (d, 6.7)	0.80 (d, 6.6)	0.78 (d, 6.3)	0.79 (d, 6.5)
C-30 substituent						
NH	6.00 (br s)	5.88 (br t, 5.4)	6.36 (d, 7.7)	6.25 (d, 7.4)	6.80 (br d, 6.9)	6.65 (br m)
NCH_n	3.30 (2H, m[b])	3.33 (2H, br q, 6.1)	4.69	4.75	4.63	4.65
			(1H, dt, 7.4, 4.9)	(1H, dt, 7.8, 4.7)	(1H, br q, 5.6)	(1H, br dt, 6.9, 4.6)
SCH_A	3.30 (m[b])	3.37 (m[b])	3.66	3.83	3.58 (m[b])	3.74
			(dd, 4.7, 15.4)	(dd, 4.4, 14.3)		(dd, 5.0, 14.7)
SCH_B	2.88 (m)	2.95	3.25	3.33	3.27	3.27
		(dt, 13.2, 6.6)	(dd, 5.2, 14.4)	(dd, 5.1, 14.4)	(br d, 13.8)	(dd, 4.4, 14.3)
$COCH_3$	1.88 (s)	1.88 (s)	1.87 (s)	1.91 (s)	1.90 (s)	1.90 (s)
CO_2CH_3	—	—	3.41 (s)	3.41 (s)	—	—

[a] Chemical shifts (δ) with $CHCl_3$ as reference, multiplicities and coupling constants (Hz), for solutions in $CDCl_3$ at 300 MHz.
[b] Adjacent signals overlapping. [c] Assignments may be interchanged.

ansamycins into thioansamycins in bacterial fermentation media probably occurs without the involvement of enzymes, and simply reflects the availability of appropriate thiol reactants in the medium. Furthermore, although the chlorine substituent facilitates the introduction of sulfur substituents into the quinones by providing an addition-elimination pathway, its presence may not in general be necessary. There is evidence that formation of the thiazinone and thiazole rings of rifamycins verde, P and Q is initiated both *in vitro* and in fermentations by the non-enzymic addition of L-cysteine to the unchlorinated naphthoquinone rifamycin S.[19~21] In these cases, re-oxidation of the resulting hydroquinone then precedes cyclisation and subsequent transformation

to the isolated antibiotics.

Experimental

General

NMR spectra were recorded on Varian Gemini-300, VXR-300S or VXR-500S spectrometers, infrared spectra on a Perkin Elmer 683 spectrometer, optical rotations on a Perkin Elmer 241 polarimeter, and UV spectra on a Hewlett Packard 8450A UV/VIS spectrophotometer. MS data were obtained on Fisons Instruments VG ZAB2-SEQ, AutoSpec and Quattro II spectrometers. Melting points were recorded on a Kofler hot stage apparatus and are uncorrected.

Physical properties of the isolated naphthomycins are recorded in Table 1, ^{13}C and ^{1}H NMR data in Tables 2 and 3. Known naphthomycins were identified by comparison of ^{13}C and ^{1}H NMR data with that reported in the literature. Yields of naphthomycins from small scale fermentations were variable.

Supplementation and Extraction of Fermentations

Streptomyces sp. E/784 was grown in 250 ml baffled Erlenmeyer flasks, each containing a medium (100 ml) prepared from dextrose 50 g, meat extract 4 g, peptone 4 g, soybean flour 10 g, yeast extract 1 g, $CaCO_3$ 5 g and NaCl 2.5 g made up to 1 liter with distilled water, on a rotary shaker (220 rpm) at 28°C.

Fermentations yielding naphthomycins (**5**) and (**6**) were supplemented with a methanolic solution (100 μl/flask) of the *N*-acetylcysteamine thioesters (**11**) or (**12**) (47 mg/700 μl) at 42, 50, 58, 66, 74, 82 and 90 hours, and harvested after 100 hours. Fermentations supplemented with a methanolic solution (150 μl/flask) of *N*-acetyl-L-cysteine methyl ester (100 mg/ml) at 24 and 48 hours afforded naphthomycins (**7**) and (**8**) when harvested after 72 hours. Supplementation with *N*-acetyl-L-cysteine using this latter protocol generated naphthomycins (**9**) and (**10**).

The flasks of each experimental set were combined and worked up as described for a set of 5 flasks. The mycelium was separated by centrifugation (10 minutes, 1500 rpm) and the supernatant decanted. Me_2CO (200 ml) was added to the cell mass and the mixture shaken vigorously for 2 minutes. After adding CH_2Cl_2 (250 ml), the mixture was again shaken and the organic layer separated. The culture supernatant was extracted with EtOAc at fermentation pH (3 × 200 ml) and again after acidification (dilute HCl) to pH 2 (3 × 200 ml). Separation of emulsified liquid phases was facilitated by centrifugation.

Isolation of the Neutral Naphthomycins (3~6), (7) and (8)

The neutral naphthomycins (**3~6**), (**7**) and (**8**) were isolated from extracts of the cell mass and the fermentation liquor before pH adjustment, which were combined, dried (Na_2SO_4) and evaporated under reduced pressure.

Naphthomycins H (**3**) and A (**4**) were purified by preparative TLC on silica gel (30% EtOAc/toluene). The two purple bands were separately scraped from the plate, extracted from the silica with methanol, and the solvent removed by rotary evaporation. The resulting purple residues were dissolved in water, acidified with a few drops of dilute HCl, and extracted with chloroform until the organic fractions were colourless. The extracts were dried (Na_2SO_4) and evaporated to give the naphthomycins as yellow solids. All isolations of naphthomycins from preparative TLC plates were performed in this manner.

The novel naphthomycins (**5**) and (**6**) were purified by flash silica gel column chromatography. Unwanted metabolites were removed by eluting the column with 30% EtOAc/toluene, after which the naphthomycins were eluted with 2% MeOH/CH_2Cl_2. Further purification was achieved by precipitation from concentrated CH_2Cl_2 solutions with CCl_4.

2-Demethylnaphthomycin F (**7**) and naphthomycin F (**8**) were partially purified by flash silica gel column chromatography (0.5% AcOH/EtOAc), and further purified by preparative silica gel TLC (15% MeOH/EtOAc) and Sephadex column chromatography (EtOAc) respectively.

Isolation of the Acidic Actamycin (1) and Naphthomycins (2), (9) and (10)

The acidic naphthomycins (**1**), (**2**), (**9**) and (**10**) were obtained from extracts of the acidified broth, which were again combined, dried (Na_2SO_4) and evaporated. The residue was extracted with CH_2Cl_2 (3 × 25 ml) using sonication to aid dissolution of metabolites, and the organic extracts combined, filtered and evaporated. Actamycin (**1**) and naphthomycin D (**2**) were purified by preparative silica gel TLC (25% MeOH/CH_2Cl_2) and isolated as described above as orange-red solids. Naphthomycin J (**9**) and naphthomycin I (**10**) were partially purified by flash silica gel column chromatography (5% MeOH/0.5% AcOH/EtOAc), and further purified by precipitation ($CHCl_3$/hexane) and preparative TLC (30% MeOH/EtOAc) respectively. The ^{13}C NMR spectrum of naphthomycin I (**10**), recorded in D_5-pyridine for comparison with the published spectrum of

naphthomycin G,[10] showed only 41 clear signals at δ 201.1, 197.9, 181.9, 179.4, 173.5, 171.4, 169.7, 159.6, 147.7, 145.1, 141.0, 140.2, 138.0, 137.7, 136.3, 134.1, 133.2, 133.0, 132.1, 130.0, 127.9, 126.8, 125.2, 78.3, 72.5, 71.7, 53.5, 46.6, 43.4, 41.7, 37.2, 36.5, 29.9, 22.9, 20.8, 18.6, 18.2, 17.2, 13.4, 12.3, and 11.7, the remaining 4 signals being superimposed on others, obscured by solvent, or too weak to detect.

Semisynthesis of the Naphthomycin (**6**) and Naphthomycin I (**10**)

Naphthomycin A (**4**) (7 mg), with spectroscopic data identical to that reported,[10,12,13] was stirred with *N*-acetyl cysteamine (6 mg, 5 equiv) in water at room temperature and pH 8 for 24 hours. Acidification and extraction with CH_2Cl_2 gave a major product as shown by TLC, which when isolated by flash silica gel column chromatography (0.5% AcOH/EtOAc) was identical to the novel naphthomycin (**6**) by ^1H NMR spectroscopy. Similar treatment of naphthomycin A (**4**) (34 mg) with *N*-acetyl-L-cysteine (39 mg, 5 equiv) in water (60 ml) at pH 8 for 5 days afforded starting material, together with naphthomycin I (**10**) (18 mg, 48%) purified by chromatography as above, identical with material obtained from fermentation.

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