The structures of premithramycinone and demethylpremithramycinone, plausible early intermediates of the aureolic acid group antibiotic mithramycin

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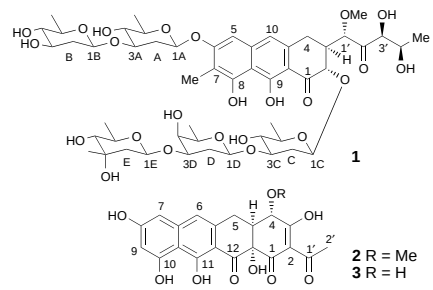
The structures of premithramycinone and its demethyl analogue suggest that the aureolic acid antibiotics are biosynthetically formed *via* a tetracycline-type, and not a tetracenomycin-type, folded decaketide.

Mithramycin[1] **1** (aureolic acid, plicamycin, mithracin, LA-7017 *etc*.[2]) and related aureolic acid group antibiotics, such as the chromomycins, olivomycins and UCH9, are important anticancer agents, effective against various experimental and human tumours.[1–6] Mithramycin in particular has been clinically used for the treatment of certain tumours as well as for Paget's bone disease.[7,8] For its mechanism of action, a Mg^{2+} dimer complex binding to GC-rich DNA regions has been investigated.[9]



Incorporation experiments on chromomycin A_3 with various single and double ^{13}C labelled acetates by Montanari and Rosazza revealed that all carbon atoms of the aglycon moiety of the aureolic acid group antibiotics are acetate, *i.e.* polyketide, derived.[10] Because of the unexpected incorporation pattern, hypotheses were raised in which two or three independent acetate chains are condensed to form the aglycon.[10] Recent molecular biology experiments[11,12] confirmed our earlier hypothesis[13] that the entire aglycon backbone of such compounds derives from a single decaketide chain. To explain Rosazza's acetate incorporation pattern, a tetracenomycin-type intermediate was suggested.[11–13] Therefore, the aureolic acid group was proposed to be biosynthetically closely related to the tetracenomycins. The tetracenomycins, produced by *Streptomyces glaucescens*, are unique among the polycyclic aromatic polyketides insofar as the formation of their tetracyclic ring skeleton requires a folding of the hypothetical decaketide intermediate leading to a first cyclization between carbons 9/14, instead of the more typical first cyclization between carbons 7/12 which is found, *e.g.* for anthracyclines, angucyclines and tetracyclines.[13–15]

Premithramycinone **2** and its demethyl analogue **3** (in smaller amounts) are accumulated by the disrupted M7D1 mutant of *Streptomyces argillaceus* ATCC 12956, which is a mithramycin producer. In the mutant M7D1 the *mtmD* gene encoding a glucose-1-phosphate : thymidylyl transferase[16,17] was inactivated through the insertion of an apramycin resistance cassette into a unique *Bam*Hl site of this gene and through gene replacement of the wild type region of the chromosome by the *in vitro* mutated one.[16] Glucose-1-phosphate : TTP thymidylyl transferase is a key enzyme of the deoxysugar biosynthesis. As a consequence, no sugar substrates for the glycosyl transfer steps in the mithramycin biosynthesis can be provided, and an aglycon substrate can be expected to be accumulated in such a mutant strain, as was discussed previously.[16] However, a wrong structure was initially suggested for premithramycinone.

The (revised) structures of **2** and **3** were determined on various physicochemical data, in particular on one- and two-dimensional ^1H and ^{13}C NMR spectra (Tables 1 and 2, Fig. 1).[18] Fig. 1 shows the $^nJ_{C,H}$ couplings (n = 2,3; HMBC) found for **2** (for **3** analogously), including the key couplings supporting these structures *vs*. the formerly suggested premithramycinone structure which can be observed between 2′-CH_3/1′-C=O and 2′-CH_3/C-2. The stereochemistry shown in **2** and **3** is suggested taking into consideration the observed H,H and C,H couplings

Table 1 ^1H NMR data (MeOH) for **2** and **3**

	δ (multiplicity, *J*/Hz)	
Proton	**2**[a]	**3**[b]
4-H	4.00 (br d, 11)	4.00 (br d, 11)
4-OCH_3	3.54 (br s)	—
4a-H	2.68 (ddd, 11, 4.5, 3)	2.50 (m)
5-H_a	3.48 (ddd, 16.5, 4.5, 1.5)	3.46 (br d, 16)
5-H_e	3.10 (dd, 16.5, 3)	3.19 (br d, 16)
6-H	6.93 (dd, 1.5, 0.5)	6.89 (s)
7-H	6.54 (dd, 2.5, 0.5)	6.52 (d, 2)
8-H	6.36 (d, 2.5)	6.33 (d, 2)
2′-H_3	2.63 (s)	2.65 (s)

[a] 300 MHz. [b] 400 MHz.

Table 2 ^{13}C NMR data (MeOH) **2** and **3**, multiplicities from APT and C,H COSY experiments

	δ (multiplicity)			δ (multiplicity)	
Carbon	**2**[a]	**3**[b]	Carbon	**2**[a]	**3**[b]
C-1	196.4 (s)[c]	196.7 (s)[c]	C-9	102.9 (d)	102.8 (d)
C-2	112.2 (s)	111.6 (s)	C-10	161.2 (s)	161.0 (s)
C-3	167.8 (s)[c]	167.4 (s)[c]	C-10a	108.1 (s)	108.1 (s)
C-4	78.7 (d)	70.0 (d)	C-11	164.0 (s)[c]	163.8 (s)[c]
C-4a	45.5 (d)	46.6 (d)	C-11a	108.1 (s)	107.9 (s)
C-5	27.0 (t)	26.7 (t)	C-12	197.4 (s)[c]	197.6 (s)[c]
C-5a	135.7 (s)	135.5 (s)	C-12a	80.0 (s)	78.9 (s)
C-6	118.8 (d)	119.0 (d)	C-1′	202.0 (s)	203.6 (s)
C-6a	143.4 (s)	143.3 (s)	C-2′	30.7 (q)	30.7 (q)
C-7	103.7 (d)	103.6 (d)	OCH_3	61.6 (q)	—
C-8	164.0 (s)	163.9 (s)			

[a] 125.7 MHz. [b] 100.6 MHz. [c] Interchangeable due to tautomerisation.

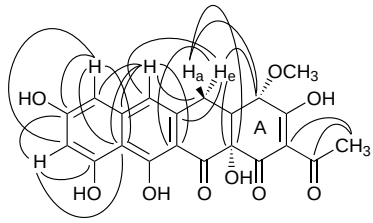
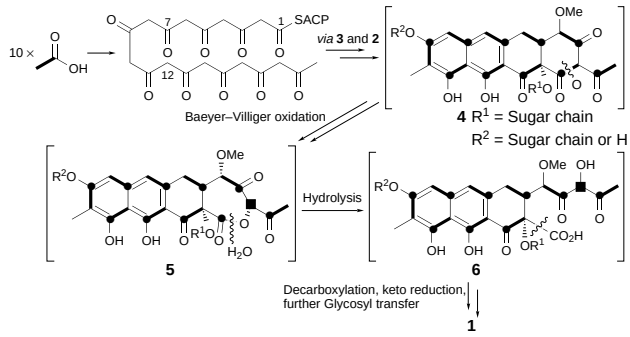


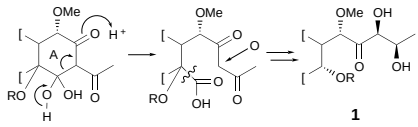
Fig. 1 $^{2,3}J_{C,H}$ Couplings observed in the HMBC spectra of **2** and **3**. Several theoretical couplings in ring A are not detectable due to tautomerisation

(Table 1, Fig. 1) and the given stereochemistry of **1** along with the conversion mechanism involving a Baeyer–Villiger type oxidation (Scheme 1). The structures **2** and **3** resemble certain tetracyclines, *e.g.* the aglycon moiety of chromocyclomycin,[19] BMS-192548 and others.[20]



Scheme 1

Thus, **2** is plausibly the substrate for the first glycosyl transfer reaction, and its glycosylated derivative[21] is likely to be the substrate for an enzyme responsible for a C–C bond scission leading to the typical tricyclic aureolic acid chromophore. For this cleavage reaction, we suggest here also[22] a Baeyer–Villiger type oxidation of **4** leading to the ε-lactone containing intermediate **5**. Its subsequent hydrolysis to **6** along with a decarboxylation reaction and eventual further glycosylation reaction leads to **1**. As an alternative, a retro-aldol-type cleavage and a subsequent oxidation of the resulting side chain (Scheme 2) may also be taken into consideration.[23] Both mechanistic sequences (Schemes 1 and 2) are also in agreement with Rosazza's and our own ^{13}C labelled acetate incorporation pattern.[10,24]



Scheme 2

The studies described here show the (presumably oxidative) C–C bond cleavage of a tetracyclic compound to be the biosynthetic key step leading to the aglycon skeleton of the aureolic acid group antibiotics, as was suggested earlier.[11–13] However, the tetracyclic compounds **2** and **3**, which are presumably biosynthetic intermediates of mithramycin, are structurally related to the tetracyclines, and not to the tetracenomycins. Thus, a tetracycline-type folding (Scheme 1) rather than a tetracenomycin-type folding and a standard first cyclase reaction between carbons 7/12 of the hypothetical decaketide intermediate (Scheme 1) is also true in the biosynthetic formation of the aureolic acid antibiotics, and all former hypotheses involving a tetracenomycin-type intermediate[12–15] have to be corrected, including the previously suggested wrong structure for premithramycinone.[16]

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Notes and References

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18 *Selected data* for **2**: *m/z* (EI-MS) 414 (M^+, 64%), 258 (100), 241 (25), 229 (22), 216 (20), 156 (72); λ(MeOH)/nm (ε) 230 (27 400), 276 (49 700), 323 (7700), 411 (13 100); λ_{extr}(MeOH)/nm (θ) 232 (−15 400), 284 (158 000), 412 (−23 800); ν_{max}(KBr)/cm^{-1} 3408, 2923, 1672, 1636, 1449, 1401, 1343, 1161, 1096, 1013, 996, 873, 743, 609. For **3**: *m/z* (EI-MS) 400 (M^+, 8%), 259 (61), 241 (48), 230 (100), 133 (90).

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24 Routine feeding experiments on *S. argillaceus* using [1-^{13}C]- and [2-^{13}C]-acetate resulted in an incorporation pattern in mithramycin identical to that found earlier in chromomycin A_3 (ref. 10).

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