The biosynthesis of pramanicin: origin of the carbon skeleton

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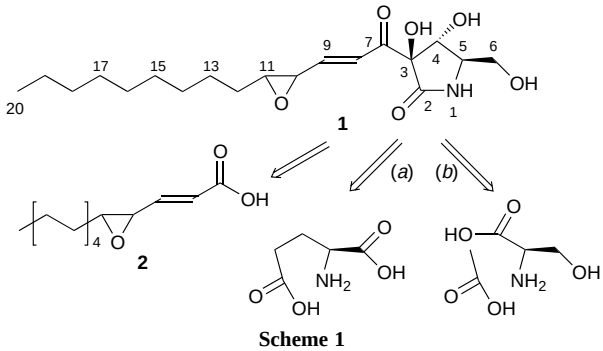
Incorporation of labelled acetates and serine into pramanicin 1 in *Stagonospora* sp. ATCC 74235 shows that the carbon skeleton of 1 is derived from eight acetate units and a serine residue, implying that the biosynthesis of 1 proceeds *via* an acyl–tetramic acid intermediate 3.

Pramanicin **1** (Scheme 1), an inhibitor of *Cryptococcus neoformans*, and the closely related fatty acid epoxide **2** were isolated by Schwartz *et al.*[1] from a sterile fungus which has subsequently been identified as a species of *Stagonospora*. Our biomimetic template-directed synthesis of **2**[2] was based on a presumed biosynthetic pathway in which seven acetate units combine in a manner typical of fatty acids and polyketides[3] to give tetradeca-2,4-dienoyl-CoA; however, the biosynthetic origin of the pyrrolidone ring in **1** is not clear. Thus, this polar headgroup could be derived from a suitable five-carbon amino acid precursor such as proline or glutamate *via* pyroglutamate and a further Claisen-like condensation onto the CoA ester of fatty acid **2** [Scheme 1, path (*a*)]. On the other hand, **1** could also be viewed as being related to the tetramic acid family of natural products which contains the 3-acyl-Δ^3-pyrrolin-2-one moiety. Biosynthetic studies on erythroskyrine,[4] β-cyclopiazonic acid,[5] tenuazonic acid,[6] malonomicin,[7] streptolydigin,[8] ikarugamycin,[9] the cytochalasans,[10] and recently a preliminary report on aflastatin A[11] have shown that the five atoms of the pyrrolinone ring in these metabolites derive from C-1 and C-2 of one acetate unit and the carboxy- and α-carbon as well as the nitrogen of the appropriate amino acid (Val, Trp, Ile, 2,3-diaminopropanoate, β-methyl-Asp, ornithine, Trp and Ala, respectively). Tetramic acid intermediates have also been proposed in the biosynthesis of, for example, tenellin[12] and ilicicolin H.[13] Thus another potential pathway to **1** would involve the formation of a linear octaketide and cyclization with, presumably, serine as the amino acid [Scheme 1, path (*b*)]. Therefore we sought to determine which pathway was operational in formation of **1**, and describe herein the results of preliminary labelling experiments which establish the origin of the carbon skeleton of **1**.

Cultures of *Stagonospora* sp. ATCC 74235 were grown, and pramanicin was isolated with minor modifications† of the literature procedure.[1] When sodium [1-^{13}C]acetate was added to the cultures, enhanced signals in the {^{1}H}^{13}C NMR spectrum were observed in the resulting **1**, compared with an unlabelled control sample (Table 1). Labelling was observed at the unambiguously assigned sites 2, 7, 9, 11, 13 and 19, as well as at two other sites within the heavily overlapped region corresponding to C-14 to C-18. Therefore, eight acetate units are incorporated into **1**. When sodium [1,2-$^{13}C_2$]acetate was incorporated into **1**, the pulsed field gradient 2D INADEQUATE NMR spectrum of the resulting sample showed seven of the eight expected couplings within acetate-derived units (Table 1).†



Scheme 1

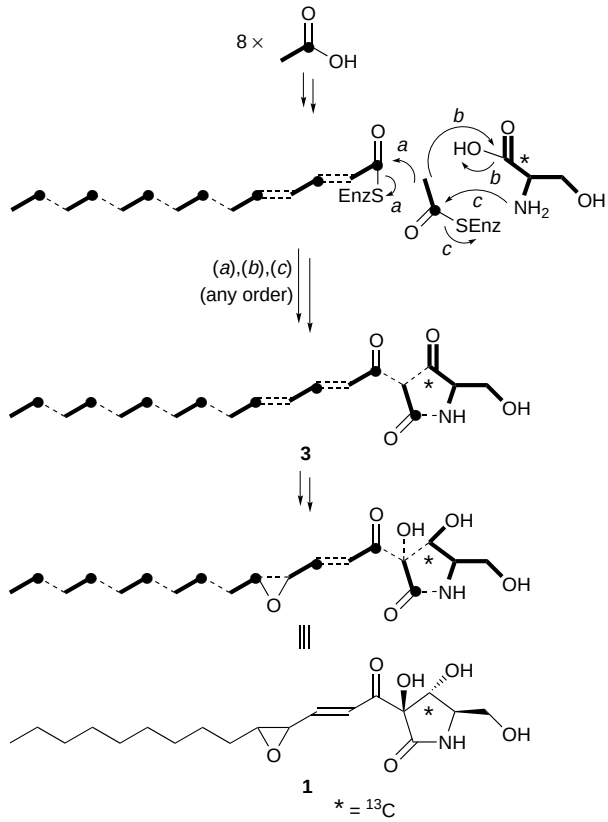
These results show that the 4,5-epoxytetradec-2-enoyl hydrophobic tail of **1** is indeed derived from the expected 'head-to-tail' condensation of seven acetate units,‡ and at first sight imply a further cycle of chain extension on the fatty acid synthase to give a sixteen-carbon β-ketoacyl chain. However, acetate can enter the tricarboxylic acid cycle and can label glutamate and hence proline: [1-^{13}C]- and [1,2-$^{13}C_2$]-acetates give [5-^{13}C]- and [4,5-$^{13}C_2$]-glutamates, respectively, which are expected to label C-2 and C-2,3 of pramanicin if path (*a*) is operational. Hence, exactly the same labelling pattern is predicted for the direct chain extension pathway as is expected for indirect incorporation *via* glutamate, and these experiments cannot distinguish between the two proposed putative biogenetic pathways.

Incorporation of DL-[1-^{13}C]serine proceeded efficiently: only C-4 of **1** was labelled (Table 1). Although serine can be converted to glutamate or proline, the intermediacy of acetate is

Table 1

		Incorporation			Coupling	
Carbon[a]	δ_C[b]	[1-^{13}C]-NaOAc[c]	[1-^{13}C]-serine[c]	[1,2-$^{13}C_2$]-NaOAc[d]	*J*/Hz[e]	To[f]
7	197.91	0.81	−0.11	2.7	56	C-8
2	174.93	0.51	−0.11	3.1	52	C-3
9	145.13	0.69	−0.07	3.1	55	C-10
8	127.88	0.04	−0.07	2.5	56	C-7
3	88.10	0.06	0.04	2.6	52	C-2
4	78.99	0.02	5.24	—	—	
11	62.90	0.74	−0.10	2.6	43.7	C-12
6	62.12	0.06	0.00	—	—	
5	60.33	−0.02	0.11	—	—	
10	57.78	0.04	−0.08	2.9	55.4	C-9
12	33.02	−0.05[g]	−0.05[g]	2.5[g]	43.7	C-11
14–18	33.02				34.7	δ 30.5
14–18	30.61	0.30[g]	−0.01[g]	ND[h]	m[i]	ND[h]
14–18	30.61			ND[h]	m[i]	ND[h]
14–18	30.48	−0.03	0.03	ND[h]	m[i]	ND[h]
14–18	30.39	0.41	0.07	ND[h]	m[i]	ND[h]
13	26.92	0.60	−0.02	2.4	34.7	δ 30.5
19	23.69	0.43	0.10	2.6	34.7	C-20
20	14.38	−0.12	0.10	3.1	35	C-19

[a] The numbering system is based on ref. 1. [b] Determined in CD_3OD. [c] Expressed as number of fold increase in the height of the carbon-13 resonance relative to unlabelled **1**, with correction by standardizing the two samples using the mean peak height for all unlabelled carbons. [d] Determined by dividing the sum of the coupled peak heights by the height of the uncoupled, natural abundance peak. [e] Values which are stated to 0.1 Hz accuracy determined from a narrow-window high-resolution ^{13}C NMR experiment. [f] Coupled partner from 2D gradient INADEQUATE experiment. [g] Values averaged over two overlapping carbons. [h] ND = not determined. [i] Multiplet; coupling patterns could not be deduced in this region.



Scheme 2

required in this process, and label is expected to appear at C-5 of **1**. Since no incorporation into acetate-derived sites was observed in this experiment, glutamate and proline cannot be precursors of the pyrrolidone moiety in **1**. Rather, the results are consistent with formation of a linear octaketide; amide formation with the amino group of serine and cyclization by formal Claisen-like condensation of the β-ketoacyl enolate onto the presumably activated serine carboxy group would generate **3**, which contains the carbon skeleton and ring of **1** (Scheme 2). The order of these events [events (*a*), (*b*) and (*c*) in Scheme 2] remains to be established. Net addition of water across the C-3–C-4 bond, presumably by oxidative hydroxylation at C-3 and reduction at C-4 of intermediate **3**, and epoxidation gives **1**. Further investigation of this pathway is being undertaken, and will be reported in due course.

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Footnotes and References

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† *Stagonospora* was cultured in liquid medium LCM,[1] with the glucose content reduced to 40 g l^{-1} (100 ml in each of twelve 500 ml Erlenmeyer flasks). Sodium [1-^{13}C]- and [1,2-$^{13}C_2$]-acetate (1 g each) or DL-[1-^{13}C]-serine (0.25 g) were added as sterile solutions in water at 24 h intervals over days 2–6. After seven days, the culture was worked up as previously described.[1] Final purification was by MPLC on a Merck LOBAR RP-8 column in MeOH–H_2O (70 : 30), giving 75 mg of **1**.

‡ The remaining correlation for the eighth unit corresponds to two carbon nuclei at *ca.* δ 30; these are strongly coupled and thus this correlation is not observed in the INADEQUATE spectrum. Careful examination of the 1D spectra shows that the two units C-17,18 and C-15,16 are each derived from an *intact* acetate unit; however, the *direction* of these units, implied in Scheme 2 in accord with biosynthetic convention,[3] is not unambiguously proven by these experiments.

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