Biosynthesis of XR587 (streptopyrrole) in *Streptomyces rimosus* involves a novel carbon-to-nitrogen rearrangement of a proline-derived unit

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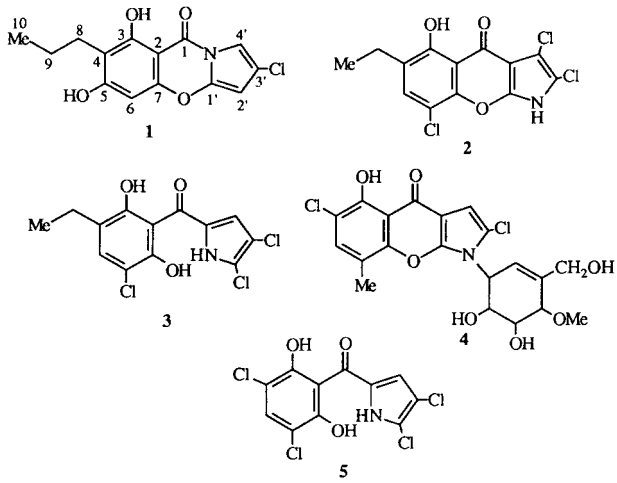
Results from incorporation studies with ^{13}C-labelled acetate, propionate and proline establish a polyketide origin for the pyrrole-containing streptomycete metabolite, XR587, and are in accord with the formation of an amide bond *via* an unprecedented rearrangement of a proline-derived starter unit.

A pyrrole-containing metabolite, XR587, was isolated from a streptomycete strain (X10/78/978; NCIMB 40808) identified as *Streptomyces rimosus* during a screening programme for antibacterial agents.[1] The novel benzopyranopyrrole structure **1**

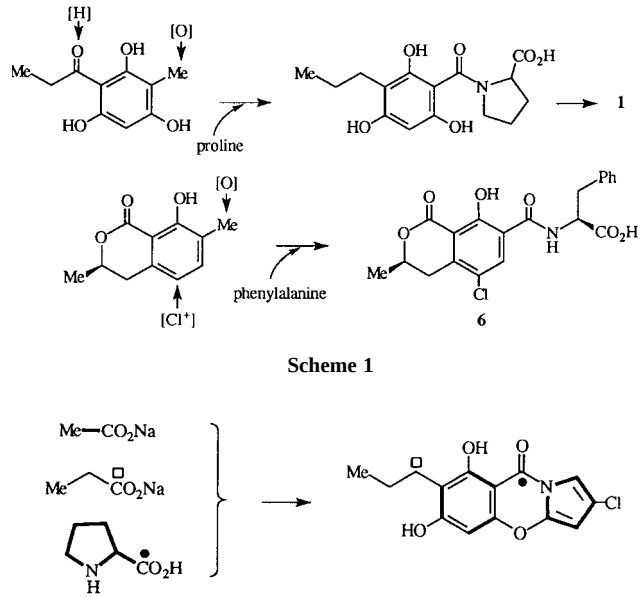


was deduced from spectroscopic studies and confirmed by X-ray crystallography. Recently, Breinholt and co-workers reported the isolation of a metabolite, streptopyrrole, from *Streptomyces armeniacus* whose structure is clearly identical to XR587.[2] Streptopyrrole was reported to display weak growth inhibitory activity against a range of fungi and bacteria.

A benzopyranopyrrole skeleton was first reported[3] for the metabolite TAN-876A **2** which is isolated along with the closely related TAN-876B **3**. The carbonyl group is, however, joined to the pyrrole unit at the β-position in **2**, and not through the nitrogen as in XR587. The pyralomycins isolated from *Microtetraspora spiralis* also contain a benzopyranopyrrole structure[4] and preliminary biosynthetic studies on the major metabolite, pyralomycin 1A **4**, indicated that the 2-ketopyrrole moiety is derived from proline although no mechanism for its formation was suggested.[5]

We initially proposed a biosynthetic pathway to XR587 in which the phenolic ring could be polyketide in origin and the pyrrole ring derived from proline. Oxidation of a propionate- (or possibly methionine-) derived methyl followed by amide formation (as indicated in Scheme 1) and subsequent oxidative modifications and chlorination would give XR587 by a pathway analogous to the biosynthesis of the mycotoxin, ochratoxin A **6**, which is formed *via* a similar polyketide linked to phenylalanine by an amide bond.[6] We now report the results of biosynthetic studies with ^{13}C-labelled precursors which show that XR587 is formed *via* two polyketide chains and a unique carbon-to-nitrogen rearrangement of proline.

The results of incorporation of [1,2-$^{13}C_2$]acetate, [1-^{13}C]propionate and [$^{13}C_5$]proline are summarised in Fig. 1 and Table 1.



Scheme 1

Fig. 1 Labelling studies on XR587 **1**.

Table 1 ^{13}C–^{13}C couplings and enrichments observed in the ^{13}C NMR spectra of XR587 **1** enriched with ^{13}C-labelled precursors

		J/Hz		
Carbon	δ_C	[1,2-$^{13}C_2$]-Acetate	[$^{13}C_5$]-Proline[c]	[1-^{13}C]-Propionate enrichment(%)
1	159.8	—	5.5, 1.5	0.7
2	93.4	65.7[a]	—	—
3	161.1	65.6[a]	2.8	—
4	113.5	68.7[a]	—	—
5	165.5	68.6[a]	—	—
6	94.4	76.3[a]	—	—
7	154.9	74.8[a]	—	—
8	25.1	—	—	54
9	22.9	—	—	—
10	14.3	—	—	—
1′	142.6	—	89.5, 11.0, 8.5, 1.5	—
2′	91.0	—	89.5, 63.5, 2.8	—
3′	119.4	84.0[b]	83.5, 63.5, 11.0, 5.5	—
4′	105.1	83.9[b]	83.5, 8.5, 2.8	—

[a] 3.2% enrichment. [b] 1.0% enrichment. [c] 5.5% enrichment.

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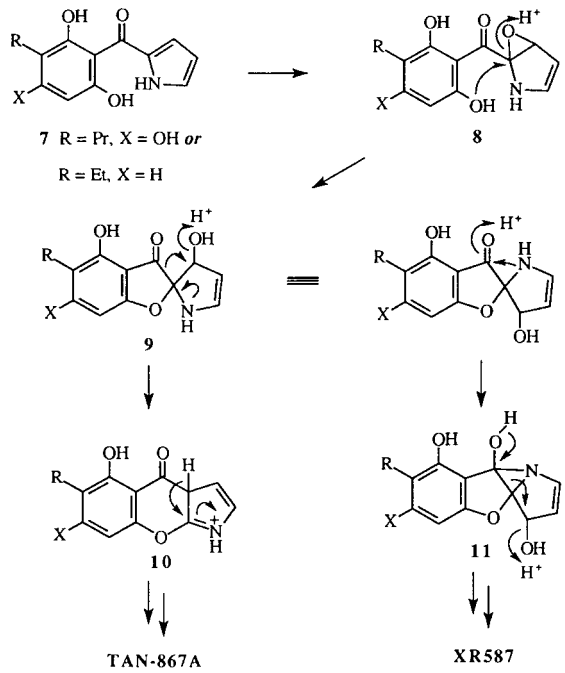


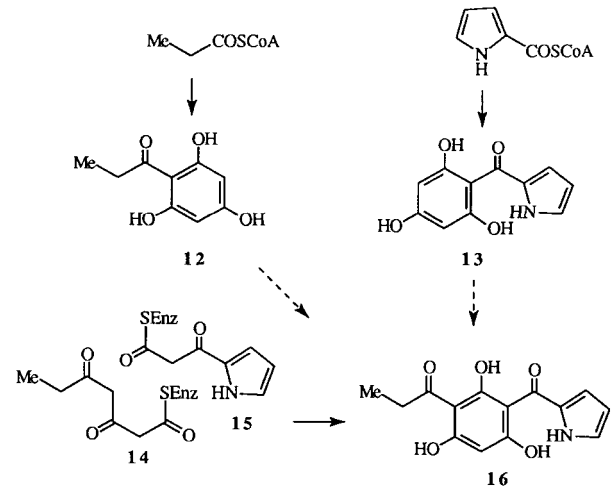
Scheme 2

The phenolic ring is derived from three intact acetate units arranged as shown in Fig. 1. The low level of labelling observed for carbons 3′ and 4′ is consistent with the derivation of the pyrrole ring from proline which itself is derived *via* α-ketoglutarate.[7] Similar low-level incorporation of acetate into the pyrrole ring of pyralomycin 1A was also observed by Kawamura *et al*.[5] An exceptionally high level of labelling from propionate (54%) was observed at C-8, and a very small amount of enrichment (< 1%) was observed at C-1. Although this low level of incorporation might have been indicative of enrichment of the C_1 pool, feeding ^{13}C-labelled methionine or formate failed to give any detectable enrichment of C-1, which implies that its derivation *via* the C_1 pool is unlikely. In fact, in several experiments, methionine was found to consistently inhibit the production of XR587. However, on feeding [$^{13}C_5$]proline, high levels of incorporation (average 5.5%) and ^{13}C–^{13}C couplings were observed at all positions in the pyrrole ring, confirming its derivation from proline. More surprisingly, the C-1 amide carbonyl was also enriched to a similar level and geminal and vicinal couplings were observed to C-1′ and C-3′ to prove that all five proline carbons are incorporated as an intact unit during the biosynthesis of XR587. Thus the original proposal for a polyketide origin for C-1 is firmly ruled out.

The pyrrolomycins, *e.g.* **5**, are pyrrole-containing metabolites of *Streptomyces fumanus* which have been shown to be derived *via* a tetraketide in which a proline-derived starter unit is extended with three acetates.[8] This, along with the reported derivation of pyralomycin 1A **4** from proline, one propionate and two acetates, and the co-occurrence of TAN-876A **2** and TAN-876B **3**, leads us to propose the pathway shown in Scheme 2 which rationalises the formation of the different benzopyranopyrrole metabolites *via* a pyrrole-polyketide intermediate **7**.

Conversion of **7** to the epoxide **8** followed by addition of the phenolic hydroxy group leads to the key spiro-intermediate **9**.‡ Subsequent loss of the hydroxy group after protonation or other activation, migration of the carbonyl bond to give the pyrrolinium intermediate **10**, and rearomatisation leads to the ketonic structure found in TAN-876A (and pyralomycins). Alternatively, addition of the pyrrolidine nitrogen to the ketonic carbonyl generates the aziridine **11** which rearranges as shown to generate the amide. To the best of our knowledge, this rearrangement of a proline-derived moiety is unprecedented.

The formation of the phenolic intermediate **7** requires comment. In the case of pyrrolomycin and pyralomycins, the corresponding intermediates may be formed from folding and condensation of a simple tetraketide intermediate which is presumably primed by the coenzyme-A thioester of pyrrole-2-carboxylate. However, the situation with XR587 is more complex. The single acetate labelling pattern observed in the phenolic ring is incompatible with a single-chain mechanism and makes the intermediacy of the phloroglucinol intermediates **12** or **13** (Scheme 3) unlikely, as there is ample precedent to suggest that the involvement of either of these symmetrical intermediates would lead to randomisation of acetate labelling of the ring.[9] Thus formation of **16** by acylation of **12** or **13** by pyrrole-2-carboxylate or propionate respectively is unlikely, and the remaining alternative requires condensation of separately formed triketide **14** and diketide **15** chains. Such two-chain mechanisms are rare but there is precedent in *e.g.* the biosynthesis of radicinin.[10]



Scheme 3

Studies to provide further evidence for this pathway, and to establish *inter alia* the exact nature of the proline-derived starter unit, reduction of C-8 and the timing of the chlorination reactions, are in progress.

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Notes and references

‡ An equally feasible alternative would involve the pyrrole nitrogen in the opening of the epoxide to give an iminium species, which would then be attacked by the phenolic hydroxy group.

1 E. Olsen, S. J. Trew, S. K. Wrigley, L. Pairet, M. A. Hayes, S. Martin and D. A. Kau, *Int. Pat.*, WO 98/25931.
2 J. Breinholt, H. Gürtler, A. Kjaer, S. E. Nielsen and C. E. Olsen, *Acta Chem. Scand.*, 1998, **52**, 1040.
3 Y. Funabashi, M. Takizawa, S. Tsubotani, S. Tanida and S. Harada, *Takeda Kenkyushosho*, 1992, **51**, 73; *Chem. Abstr.*, 1993, **118**, 55722x.
4 N. Kawamura, R. Sawa, Y. Takahashi, K. Issiki, T. Sawa, N. Kinoshita, H. Naganawa, M. Hamada and T. Takeuchi, *J. Antibiot*, 1995, **48**, 435.
5 N. Kawamura, R. Sawa, Y. Takahashi, T. Sawa, H. Naganawa and T. Takeuchi, *J. Antibiot.*, 1996, **49**, 657.
6 A. E. de Jesus, P. S. Steyn, R. Vleggaer and P. L. Wessels, *J. Chem. Soc., Perkin Trans. 1*, 1980, 52.
7 A. L. Lehninger, *Principles of Biochemistry*, Worth Publishers, 1982, pp. 450–451.
8 G. T. Carter, J. A. Nietsche, J. J. Goodman, M. J. Torrey, T. S. Dunne, M. M. Siegel and D. B. Borders, *J. Chem. Soc., Chem. Commun.*, 1989, 1271.
9 T. J. Simpson, *Top. Curr. Chem.*, 1997, **195**, 1.
10 B. Tal, G. Goldsby, B. A. Burke, A. J. Aasen and D. J. Robeson, *J. Chem. Soc., Perkin Trans. 1*, 1988, 1283.

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