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On the biosynthesis of an inhibitor of the p53/MDM2 interaction

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Abstract—The biosynthesis of a fungal secondary metabolite, chlorofusin, which disrupts the interaction between the proteins p53 and MDM2, has been investigated; the acetogenic origin of the chromophore backbone as well as of an aminodecanoic acid residue is demonstrated.

The structure elucidation of a novel fungal secondary metabolite, chlorofusin, which disrupts the interaction between the two proteins MDM2 and p53 has recently been reported.[1] Molecules such as chlorofusin could act to restore normal function to p53 in cases where tumors arise from overexpression of MDM2,[2,3] and could therefore be useful leads in cancer therapy. Chlorofusin was isolated from a tropical insect-associated fungal strain previously assigned to the genus *Fusarium*, but following further conidiogenesis studies, this assignment has now been reviewed and updated as *Microdochium caespitosum* (=*Idriella caespitosa).* The structure of this natural product (Fig. 1) is constructed from a ring of nine amino acids connected to a chromophore. This chromophore is believed to be unique, although the backbone is structurally similar to the type of structures derived from polyketides. The chromophore backbone also bears some resemblance to the isoquinoline alkaloids, which are typically derived from tyrosine.[4] However, it has recently been shown that certain isoquinoline alkaloids are derived from acetate units via an acetate–polymalonate pathway.[5,6] We report here a study of the biosynthetic pathway leading to the chromophore of chlorofusin. This pathway has been investigated by the application of ^{13}C-labeled acetate feeding experiments and analysis by ^{13}C NMR spectroscopy. The results establish the acetogenic origin of the chromophore as well as of the carbon skeleton of the aminodecanoic acid residue.

An aqueous solution of ^{13}C-labeled sodium acetate ($CH_3{}^{13}CO_2Na$, 5 g) was added to a culture of the producing organism[1] over a period of 48 h from the end of day 2 to the end of day 4 of the production stage of the fermentation. The biomass was extracted with methanol followed by ethyl acetate, and the combined extracts were then dried with magnesium sulfate. The observed isotope pattern for the $[MH]^+$ ion of chlorofusin, generated by analysis of this crude extract by LC–MS, confirmed that ^{13}C had indeed been incorporated to varying degrees. The extract was purified by preparative reverse phase HPLC using a water–acetonitrile gradient, increasing from 50 to 70% acetonitrile over a period of 12 min, then to 100% acetonitrile over a further 1 min. The column eluate was monitored at 400 nm and the major peak was collected and concentrated in vacuo to yield 9.1 mg of pure chlorofusin. The ^{13}C NMR spectrum was acquired in DMSO (Fig. 2), clearly revealing the incorporation of ^{13}C at positions 1, 3, 5, 7, 9, 11 and 17, as well as at alternate carbons along the alkyl chain of the aminodecanoic acid residue (Fig. 3). Eight additional carbonyl resonances were also enriched with ^{13}C; four with high levels of incorporation and four with much less intense signals. They could not be assigned from the 1D ^{13}C spectrum due to severe overlap in the region between 170 and 174 ppm, but from an HMBC spectrum the four intense signals

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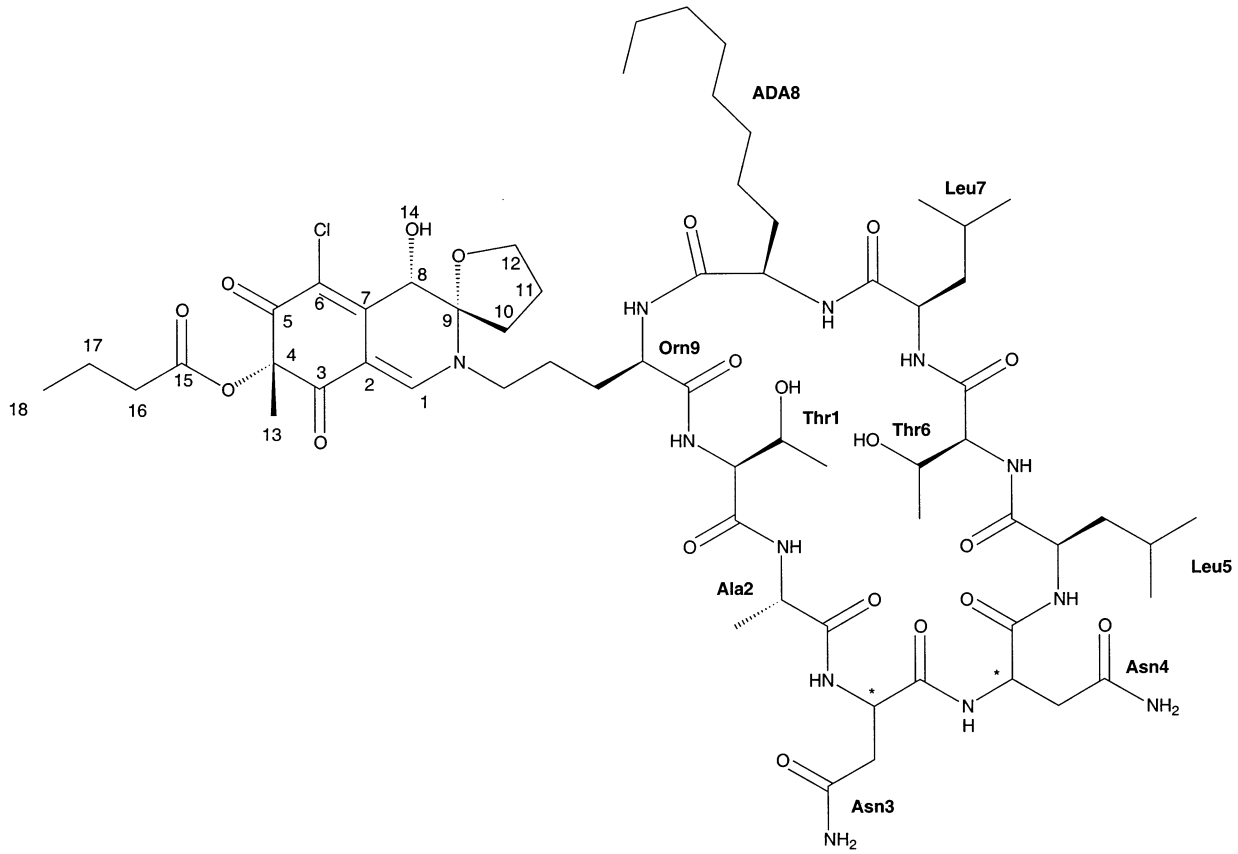

Figure 1. Structure of chlorofusin. The absolute stereochemistries for C-4, C-8 and C-9 may be as shown or with all three inverted. The two asparagine α-carbons marked with an asterix have opposite stereochemistry; one is *R* and one is *S*.

were identified as C15 in the chromophore and the carbonyls of residues Leu5, Leu7 and ADA8. Additionally, the four weaker signals could be assigned as the backbone and side-chain carbonyls of the two asparagine residues. The labeling of the carbonyl group of ADA establishes the formation of the complete carbon skeleton of this amino acid from acetate units. The observations are also consistent with proposed biosynthetic mechanisms for the common amino acids. Leucine is derived from pyruvate; its synthesis involves a condensation between α-keto isovalerate and acetyl-coenzyme-A, at which point the carbonyl carbon from acetate would be incorporated into the carbonyl position of the amino acid. This accounts for the observed high levels of ^{13}C incorporation into the carbonyl positions of the two leucine residues.[7] The labeling of the asparagine carbonyls can be explained by the transfer of ^{13}C from acetate around the glyoxylate cycle, forming malate which is then converted into oxaloacetate, the precursor for asparagine synthesis. In this way, the asparagine carbonyl carbons would become labeled with ^{13}C. The incorporation of ^{13}C labels at alternate carbons around the chromophore backbone clearly demonstrates the acetogenic origin of this unit, implying its formation from the cyclisation and modification of a hexaketide (Scheme 1). The actual sequence of reactions in this polyketide folding and condensation is unknown, but numerous 'tailoring' reactions would be required to produce the final chromophore. There is a striking similarity between the chlorofusin chromophore and some of the fungal azaphilones such as rubropunctamine and monascorubramine.[8,9] It is possible that chlorofusin is formed in a similar manner to these compounds from a reaction between an acetate derived azaphilone and the ornithine of the peptide macrocycle.

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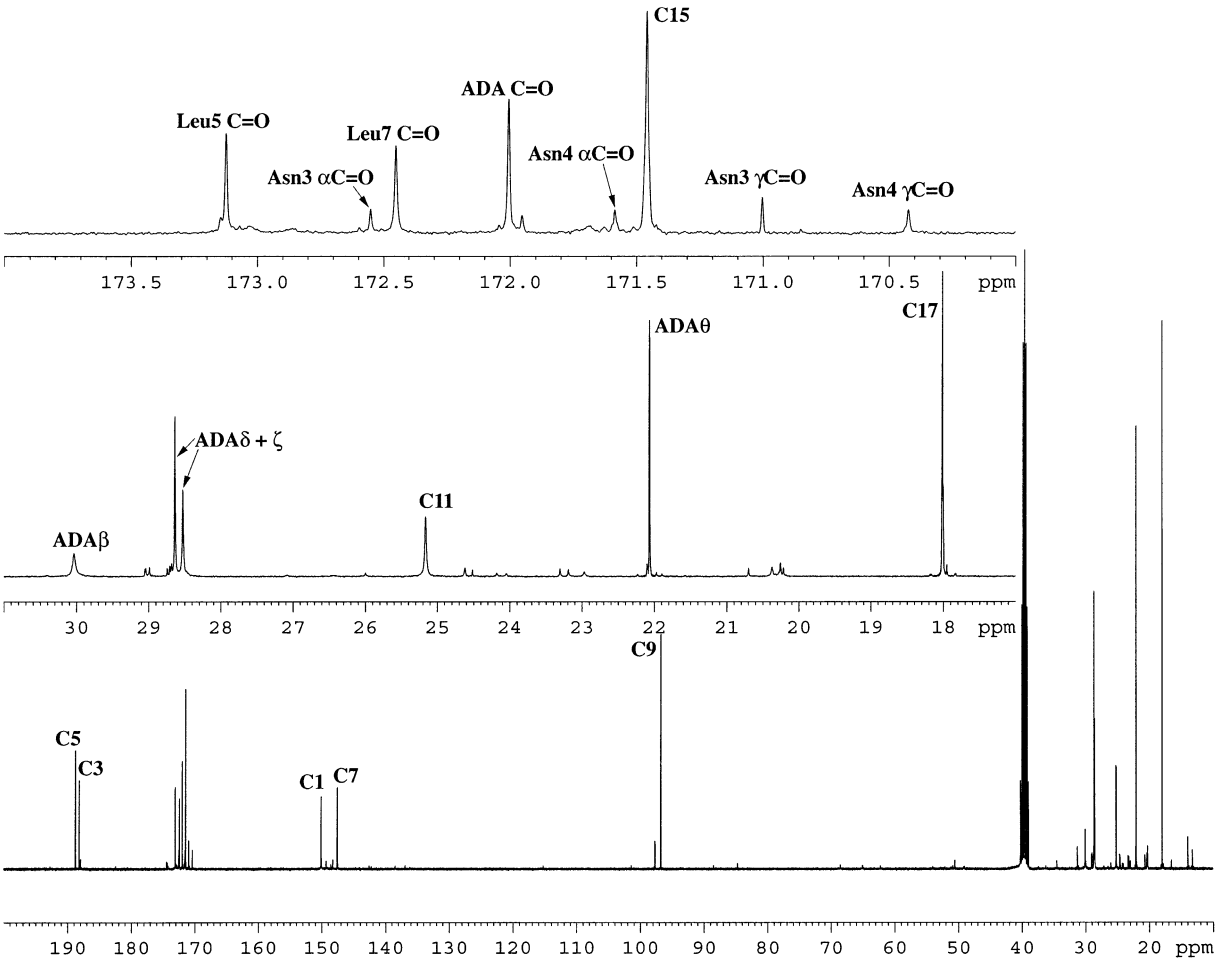


Figure 2. ^{13}C NMR spectrum (100 MHz, DMSO) of ^{13}C-enriched chlorofusin obtained after feeding $CH_3{}^{13}CO_2Na$. The inserts show expansions of the regions between 170 and 174 ppm and between 17 and 31 ppm.

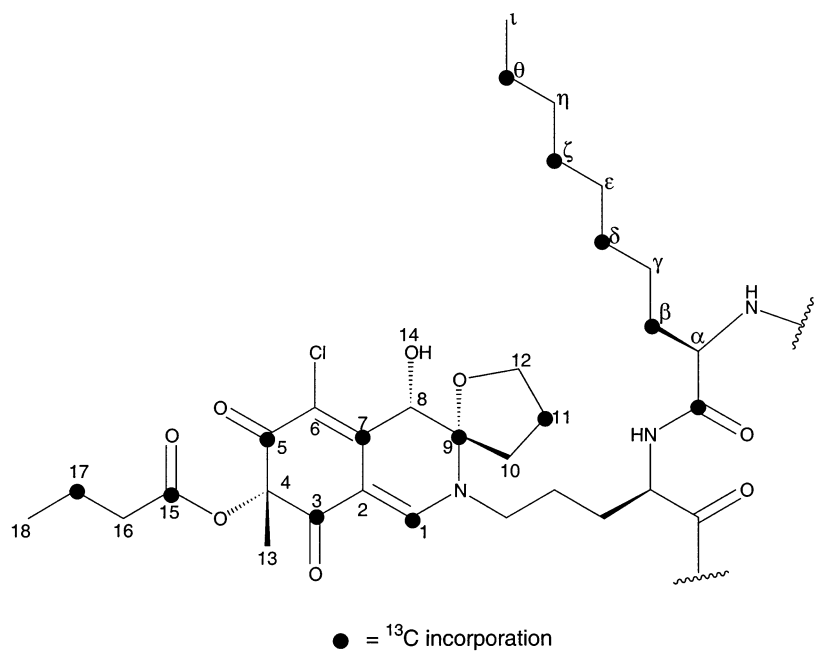
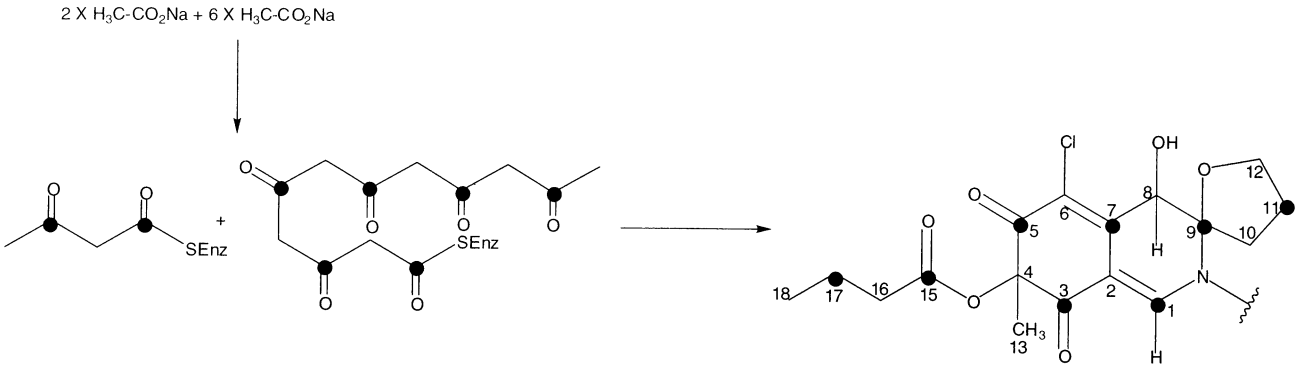


Figure 3. ^{13}C labelling pattern of chlorofusin obtained after feeding $CH_3{}^{13}CO_2Na$. ^{13}C enrichment is also observed at the carbonyl carbons of residues Leu5 and Leu7, and with a lower degree of incorporation at the backbone and side-chain carbonyls of Asn3 and Asn4.



Scheme 1.

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