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Novel jadomycins: incorporation of non-natural and natural amino acids

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Abstract—Electrospray ionization mass spectrometry of extracts from *Streptomyces venezuelae* ISP5230 cultures grown on chemically synthesized non-natural L-amino acids, D-amino acids or any of the 20 natural amino acids demonstrated incorporation of the amino acid into a jadomycin B analogue.

The need to develop new therapeutically active agents and to enlarge the reservoir of novel bioactive structural scaffolds provides impetutus to discover versatile routes to classes of secondary metabolites. *Streptomycetes* are soil bacteria responsible for producing a wide array of secondary metabolites, many of which have been developed into therapeutically important agents to treat a wide array of diseases and health-related conditions. Jadomycin was the first secondary metabolite to be isolated from *Streptomyces venezuelae* ISP5230 cultures as a result of environmental stress (e.g., ethanol/temperature/phage)[1] and a glycosylated derivative (jadomycin B) was subsequently characterized after modifying the isolation conditions (Fig. 1).[2] Jadomycins show pharmacological activity against cancer cell lines and biological activity versus bacteria and yeast.[3] The jadomycins exhibit two striking structural features: the relatively rare 2,6-dideoxysugar, L-digitoxose and a five-membered oxazolone ring generated from a polyketide-derived angucycline intermediate and an amino acid required as a metabolic nitrogen source.

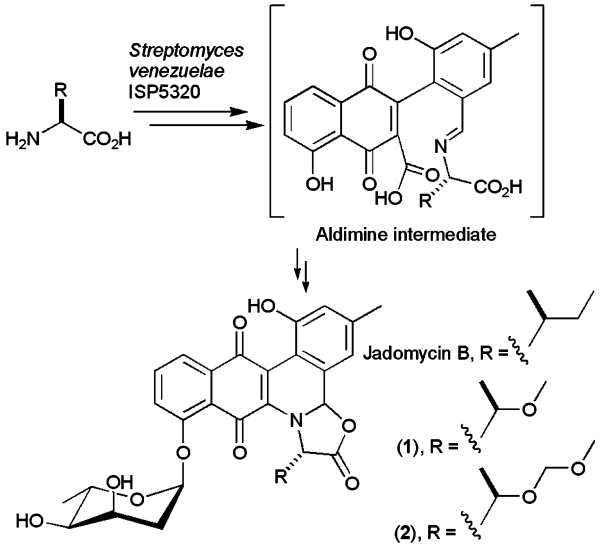

Figure 1. Secondary metabolites produced by ethanol-shocked *Streptomyces venezuelae* ISP5230.

Molecular genetic analysis of jadomycin B biosynthesis,[4–10] indicated the absence of candidate genes able to catalyze formation of the oxazolone ring, supporting a pathway in which L-isoleucine reacts spontaneously with an aldimine intermediate (Fig. 1).[11,12] Non-enzymic aldimine intermediates have also been postulated in the biosynthesis of betaxanthin in plants.[13] Forming the oxazolone ring non-enzymically could provide access to a wide variety of structural analogues, since the amino acid is used as the sole nitrogen source in the growth media, providing an amenable route to a variety of

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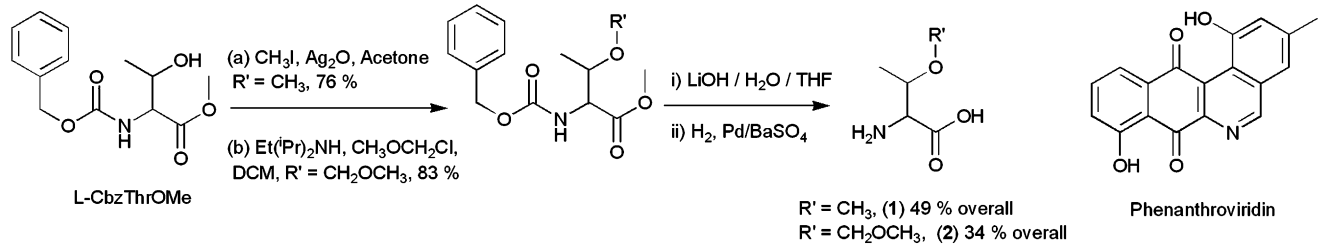

Scheme 1. Synthesis of non-natural amino acid analogues (**1**) and (**2**).

structural analogues with potentially interesting bioactivity. Altering growth conditions by using different amino acids was initially reported by Doull et al.[3] Herein, initial mass spectrometry data are presented on the incorporation of two non-natural amino acid analogues, *O*-methoxy-L-threonine (**1**) and *O*-methoxy-methylene-L-threonine (**2**), D-val, D-ile and all 20 naturally occurring L-amino acids.

The syntheses of the isosteric isoleucine analogue **1** and novel analogue **2**, starting from commercially available *N*-carbobenzyloxy-L-threonine methyl ester, are presented in Scheme 1 (1D and 2D ^{1}H and ^{13}C NMR data are included in the Supplementary data for compounds **1** and **2**).

Streptomyces venezuelae ISP5230 cultures were grown as described previously using one of the amino acid analogues as a sole nitrogen source.[2†] The cultures grew, based on doubling of optical density (OD) 600 measurements, and were shocked with ethanol after the standard 6.5 h. In comparison to growths to produce jadomycin B, the cultures were then left for an additional 24 h before harvesting and extracting due to a less intense colour build-up post ethanol shock. The delay in colour build-up may potentially arise from difficulties in metabolizing these amino acid analogues. Electrospray ionization mass spectrometry (ESI-MS) data of the infused culture extracts is presented in Table 1 and the corresponding enhanced product ion (EPI) scan data in Figure 2 (EPI mass spectra for all extracts are included in the Supplementary data). These data provide definitive evidence for the incorporation of the non-natural amino acids into the jadomycin aglycone by the observation of the parent ion ($[M+H]^+$) and the unequivocal breakdown of this ion to the non-glycosylated aglycone ($[aglycone+H]^+$) and subsequently to phenanthroviridin.

When D-Val and D-Ile were used as sole nitrogen sources, the cultures initially grew at a reduced rate on the minimal media and ethanol shock was delayed from 6.5 to 10 h, however, over the remaining 48 h the intensity of the culture colour was comparable to growths on L-amino acids. From the ESI-MS data in Table 1 it was observed that the two amino acids were incorporated into a corresponding jadomycin B analogue.[‡] As expected, the enhanced product ion spectra of these amino acid substituted jadomycins also broke down into the aglycone and ultimately the phenanthroviridin. Table 1 also summarizes electrospray mass spectral (ESI-MS) data of all ethyl acetate culture extracts from the naturally occurring L-amino acids. These data were also acquired in enhanced product ion mode providing definitive evidence of parent ions $[M+H]^+$ that are unequivocally the results of incorporating naturally occurring L-amino acids into the jadomcyin aglycone. The ESI-MS data intensities of parent ion and fragmentation peaks for the $[aglycone+H]^+$ and $[phenanthroviridin+H]^+$ observed in enhanced product ion mode were highly dependent on the collision energy between Q0 and Q2; these fragments were not observed unambigiously for all acidic or all basic amino acid extracts. The importance of the oxazolone ring upon pharmacological activity and subsequently pharmacokinetic, pharmacodynamic parameters will be reported in due course.

Table 1. Enhanced product ion scan (*m/z*), parent ions observed for ESI-MS of ethyl acetate culture extracts of *Streptomyces venezuelae* ISP5230

Amino acid	$[M+H]^+$	Amino acid	$[M+H]^+$	Amino acid	$[M+H]^+$
1	552.0	L-Ala[a]	507.3	L-Cys[a]	540.2
2	582.1	L-Gly	494.5	L-Met[a]	568.3
Jadomycin B	550.3	L-Leu	550.2	L-Asp	552.3
D-Ile	550.3	L-Val	536.4	L-Glu[a]	566.2
D-Val	536.5	L-Trp	623.2	L-Arg	593.3
L-Ser	524.3	L-Tyr	600.5	L-His[a]	574.4
L-Thr	538.3	L-Asn	551.4	L-Lys[a]	565.4
L-Phe	583.9	L-Gln	565.3	L-Pro[a]	536.2

[a] Parent ion fragmentation to the phenanthroviridin $[M+H]^+$ 306 was not observed.

The incorporation of novel non-natural amino acid analogues by *Streptomyces venezuelae* ISP5230 into jadomycin B analogues significantly broadens the potential range of secondary metabolites that can be produced

† The molar concentration of amino acid was 70 mM.[3] Strain VS1099 was used to obtain a twofold increase in production levels of the jadomycin analogue.[14] Extracted masses varied between 1 and 30 mg of material. Extracts were standardized to an $Abs_{313} = 0.01$ in 50% methanol (0.5% formic acid) prior to ESI analysis on an Applied Biosystems-MDS SCIEX triple quadrupole linear ion trap with an ambient source temperature, N_2 as collision gas for enhanced product ion scans, and with a turbospray ion source loaded by infusion at 10 μL min^{-1}.

‡ For D-val and D-ile extracts, the stereochemical assignment at the α- (and β-) carbons remains to be determined. The structure of the isolated product resulting from growth on L-pro remains to be determined and will be reported in due course.

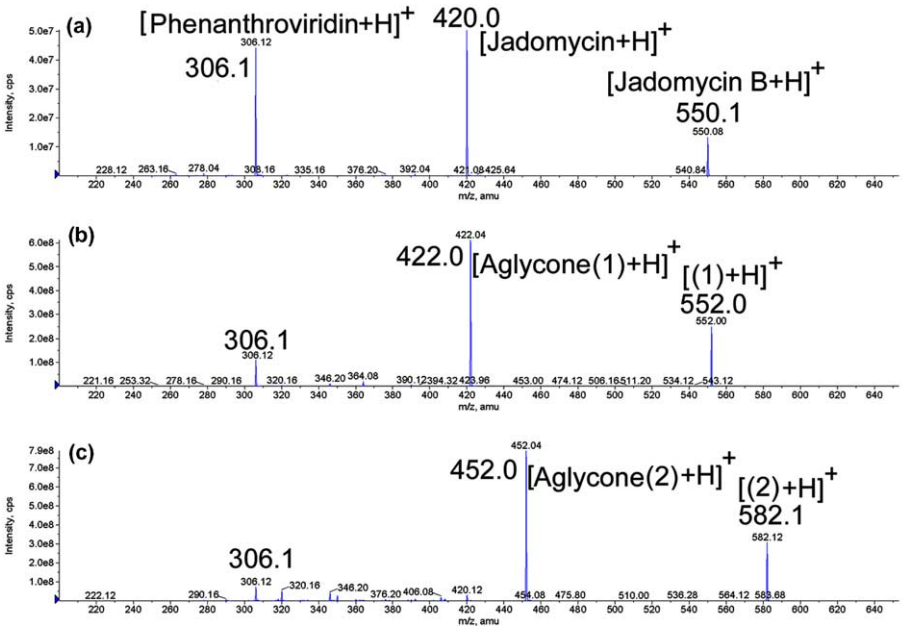

Figure 2. Representative ESI-MS enhanced product ion scan of ethyl acetate extracts of *Streptomyces venezuelae* ISP5230 cultures grown with amino-acids: (a) L-isoleucine, (b) analogue (**1**) and (c) analogue (**2**).

by *Streptomyces venezuelae* ISP5230 by complementing those jadomycin B analogues available through incorporation of natural L-amino acids. This is a significant factor in the quest for new and improved bioactive secondary metabolites and the elucidation of biochemical pathways for secondary metabolite formation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.12.082.

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