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BIOSYNTHESIS OF MACROLACTAM ANTIFUNGAL AGENTS

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Abstract: The aglycone of the macrolactam antifungal agents is biosynthesized via a combination of polyketide and TCA mechanisms.

Macrolactams are a new class of antifungal agents produced by *Actinomadura vulgaris* and *Actinomadura fulva*[1-4]. The cyclic structure of these compounds is made up of multiple numbers of saturated carbons with one or more sugars attached to one of these carbons. SCH 38516 is a representative of this class whose structure has been confirmed by X-ray crystallographic data[1]. The unique presence of a CH_2NHCO linkage,[2-7] as well as the homologous nature of the aglycone led us to explore its biosynthesis utilizing ^{13}C and ^{15}N enriched precursors.

The culture was germinated from a frozen whole broth by transferring 2.5 ml of the stock culture to 50 ml of media in a 250 ml Erlenmeyer flask, incubating at 30°C and shaking at 350 rpm for 48 hours. The germination media contained (w/v), beef extract, 0.3%; tryptone, 0.5%; yeast extract, 0.5%; cerelose, 0.1%; potato starch, 2.4%; and calcium carbonate, 0.2%. A second germination was carried out in the same medium by transferring 25 ml of the first germination into 350 ml of media in a 2 liter flask with the same incubation conditions. Fermentation was initiated by transferring 17.5 ml of the second germination into 350 ml of the fermentation medium (Bacto-Czapek Dox Broth - Difco)(in a 2 liter Erlenmeyer flask. The ^{13}C precursors (0.02 - 0.025%, w/v), 99% enriched [1-^{13}C] acetate, [2-^{13}C] acetate, [1,2-^{13}C] acetate, [2,3-^{13}C] succinate, [1-^{13}C] propionate, [3-^{13}C] propionate, L-[methyl-^{13}C] methionine, and [2-^{13}C] glycine were added to the fermentation media. One half the quantity of each precursor was added at the time of inoculation ("0" time) and at 18 hours. The culture was fermented for 72 hours at 30°C and at 350 rpm and harvested. Similar experiments were carried out with ^{15}N 99% enriched precursors, e.g., ammonium sulfate, glycine, and L-aspartic acid.

The fermentation broth (4L) was extracted with n BuOH twice (8 L each). Organic extracts were concentrated to about one liter, washed with water and dried over Na_2SO_4. Purification of this extract on a silica gel column (1 X 8") and elution with a mixture of toluene:methanol (85:15) provided the pure compounds, Sch 39185, 38516 and Sch 38518. The isolation procedure was monitored by T.L.C. (Whatman silica gel plates, developing solvent toluene:methanol 8:2 with *Rf* values of 0.2 (Sch 38518), 0.22 (Sch 39185) and 0.25 (Sch 38516)) using water and H_2SO_4 sprays.

Proton-noise decoupled ^{13}C NMR spectra of the natural abundance and the enriched macrolactams were recorded in $CDCl_3$-CD_3OD on a Varian XL-300 NMR spectrometer operating at 75.48 MHz‡.

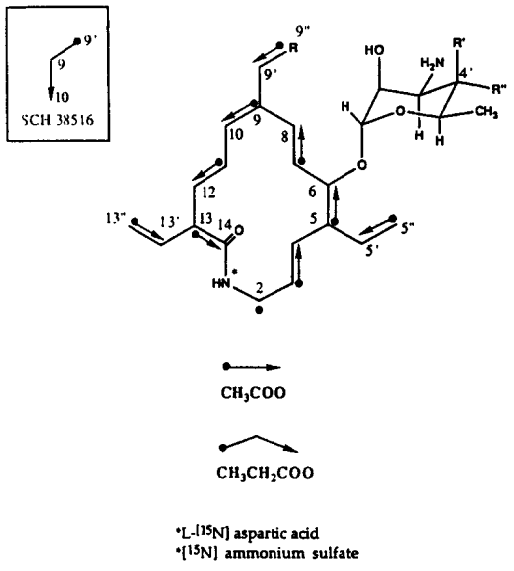
The ^{13}C NMR spectrum of SCH 39185 labeled with [1-^{13}C] acetate showed strong (>20%) enrichment of nine carbons at δ 178.3 (C-14), 77.7 (C-6), 33.9 (C-12), 32.6 (C-10), 27.7 (C-9'), 27.0 (C-13'), 25.6 (C-4), 22.8 (C-8) and 21.6 (C-5'). Similarly the spectrum derived from an [2-^{13}C] actetate enriched sample showed strong (>20%) incorporation of ^{13}C isotope for ten carbons at δ 50.6 (C-13), 41.1 (C-5), 39.0 (C-2), 38.5 (C-9), 27.9 (C-3), 24.9 (C-11), 21.7 (C-7), 12.3 (C-9"), 12.2 (C-13") and 9.0 (C-5"). The ^{13}C-^{13}C coupling pattern for C-2 (d, $^1J_{cc}$=36.8 Hz) and C-3 (d, $^1J_{cc}$=36.8 Hz) indicated that these carbons originated from [2-^{13}C] acetate. The incorporation studies with [1,2-^{13}C] acetate confirmed the above data. The acetate derived biosynthesis of the macrolactam aglycone is shown in Figure 1. The main alkyl chain of the aglycone is polyketide derived. However, the incorporation of [2-^{13}C] acetate into carbons 2 and 3 and that of [1-^{13}C] acetate into carbon 4 suggested the role of the tricarboxylic acid (TCA) cycle in the biosynthesis, e.g., a three carbon unit (C2, 3 and 4) produced by decarboxylation of a product of the TCA cycle. Indeed the enrichment experiment with [2,3-^{13}C] succinate confirmed the data obtained from [2-^{13}C] acetate, the succinate moiety being incorporated into the macrolactam aglycone after forming [2-^{13}C] acetate during the TCA cycle. A previous example of the mixed polyketide and TCA cycle mechanism has been reported[8]. No incorporation was observed when [2-^{13}C] glycine or [1-^{13}C] propionate were tried as precursors.

SCH 38516 has one less methyl group (lack of C-9") and for this metabolite ^{13}C incorporation was studied with L-[methyl-^{13}C] methionine and [3-^{13}C] propionate precursors. The former was not incorporated while the latter enriched one carbon at δ 20.6 (C-9' CH_3) indicating that carbons 9', 9 and 10 originate from propionate. **(INSERT FIG. 1)**

Since the incorporation of [2-^{13}C] actetate or [2,3-^{13}C] succinate into C-2 suggested the role of the TCA cycle in the biosynthesis, we decided to study the incorporation of ^{15}N utilizing glycine, aspartic acid and ammonium sulfate.

The proton NMR spectrum of a sample enriched from L-[^{15}N]aspartic acid in $CDCl_3$-CD_3OH showed the presence of NH at δ 7.02 (ddd, J^{15}_{NH} = 91.2 Hz, J_{HNCH} = 8.6, 3.3 Hz), which also indicated, by integration, 29% incorporation. A doublet (J^{15}_{NC} = 13.5 Hz) for the amide carbonyl was present in the ^{13}C NMR spectrum. The chemical shift of ^{15}N resonance obtained through inverse detection (HMQC) techniques at 50.68 MHz was 106 ppm (δ ^{15}N for formamide in DMSO-d_6 was taken as 112 ppm), Figure 2. When [^{15}N] ammonium sulfate was used as a precursor, the amide nitrogen was enriched by 17%. The mass spectral data of ^{15}N enriched samples supported the enrichment factors obtained from the NMR data. [^{15}N] glycine was not incorporated into the aglycone.

Thus the aglycones of Sch 38518 and Sch 39185 were biosynthesized from acetates and that of Sch 38516 from acetates and one propionate via a combination of polyketide and TCA mechanisms. The amide nitrogen originated from L-aspartic acid either via transamination or perhaps via its decarboxylation to β-alanine in the TCA cycle. Finally for the homologous macrolactam aglycones having fewer ethyl functions, e.g., lacking C-5", C-9" or C-13", the carbons 6,5,5' or carbons 14,13,13' appear to originate from a propionate precursor.



1. SCH 38518 R = -CH_3, R' = -H, R'' = -OH
2. SCH 39185 R = -CH_3, R' = -OH, R'' = H
3. SCH 38516 R = -H, R' = -OH, R'' = H

Figure 1: Biosynthesis of Macrolactam Aglycone.

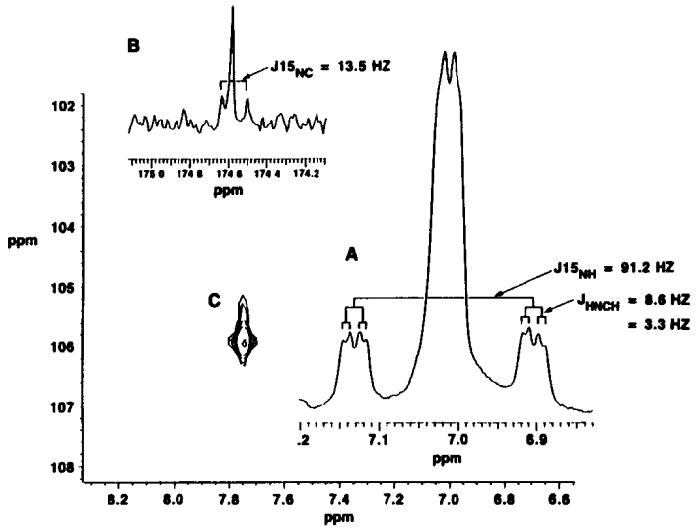


Figure 2: Incorporation studies with L-[^{15}N]aspartic acid: (a) the NH region of the proton NMR spectrum, (b) the ^{13}C spectrum showing J_{15NC} for C14, and (c) the ^{15}N resonance at δ 106.

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‡Sch 39185 aglycone ^{13}C shifts: 39.2 (C2), 28.1 (C3), 25.6 (C4), 41.2 (C5) 21.6 (C5'), 9.0 (C5"), 77.6 (C6), 21.8 (C7), 22.7 (C8), 39.2 (C9), 27.7 (C9'), 12.6 (C9"), 32.6 (C10), 25.5 (C11), 33.9 (C12), 50.9 (C13), 26.9 (C13'), 12.3 (C13"), 178.2 (C14).

Sch 38516 aglycone ^{13}C shifts: 38.2 (C2), 27.3 (C3), 24.5 (C4), 40.3 (C5), 19.8 (C5'), 8.3 (C5:), 76.0 (C6), 20.7 (C7), 24.5 (C8), 30.5 (C9), 19.8 (C9'), 33.5 (C10), 24.3 (C11), 32.8 (C12), 49.8 (C13), 26.0 (C13'), 11.5 (C13"), 176.8 (C14).
