**Note**

Isolation of (-)-14-O-Malonylindolactam-V as a Possible Precursor of (-)-Indolactam-V and (-)-14-O-Acetylindolactam-V from *Streptomyces blastmyceticum*

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(-)-Indolactam-V (1) has the fundamental structure of potent tumor-promoting teleocidins. A new teleocidin-related metabolite, (-)-14-O-malonylindolactam-V (2), was isolated from the culture broth of *Streptomyces blastmyceticum* NA34-17 which produced a large quantity of 1 along with a small amount of (-)-14-O-acetylindolactam-V (3). Heat treatment of 2 in methanol readily produced 1 and 3, suggesting that *S. blastmyceticum* NA34-17 excreted 1 as a malonic acid conjugate to accumulate 1 and 3 in the culture broth during cultivation.

**Key words:** (-)-indolactam-V; malonic acid conjugate; *Streptomyces blastmyceticum*; teleocidin; tumor promoter

Teleocidins are potent skin tumor promoters produced by actinomycetes. Among the teleocidin-producing actinomycetes, *Streptomyces blastmyceticum* NA34-17 characteristically produces a large quantity of (-)-indolactam-V (1), the common biosynthetic intermediate of teleocidins (Fig. 1). This characteristic is favorable for isolating new teleocidin-related metabolites, especially the biosynthetic intermediates of teleocidins. We have isolated several teleocidin-related compounds from this actinomycete which contributed to research on structure-activity relationships, metabolism in mammals, and teleocidin biosynthesis. Further studies to identify new teleocidin-related compounds led to the isolation of (-)-14-O-malonylindolactam-V (2). We describe here its isolation, identification, and significance in the production of 1.

Purification of 2 was guided by Ehrlich’s reagent, whereby teleocidin-related compounds showed characteristic coloration by tlc (green, blue or purple). *S. blastmyceticum* NA34-17 was cultured by deep aerated fermentation for 45 hrs (20 liters), and the concentrated culture broth (2 liters) was extracted with ethyl acetate. The extract (ca. 7 g) was chromatographed on silica gel with chloroform and increasing volume of methanol to give a 20% methanol eluate (362 mg) in which we found a new compound positive (green) to Ehrlich’s reagent of high polarity. This fraction was further purified by HPLC in a preparative C18 reversed-phase column by a linear gradient of 10-45% acetonitrile in water containing 0.1% trifluoroacetic acid. Since 2 was easily decomposed by heating, the collected fraction was concentrated in vacuo at under 30°C to remove acetonitrile and lyophilized to give 2 (10 mg) as an amorphous solid, [α]D -47.4° (c=0.305, MeOH, 23.1°C). (-)-Indolactam-V (1, 600 mg) was also isolated from this culture broth.

The molecular formula of 2 was established to be C19H18N2O by HR-FAB-MS (obs. m/z 388.1855; calc. 388.1872 for [MH+]). The presence of an indole chromophore was confirmed by the UV spectral data (λmax (MeOH) nm (ε): 226 (24,300), 298 (6,800)). The 1H-NMR spectrum of 2 in methanol-d4 (Table 1) indicates that 2 exists as two stable conformers (sofa:twist=2:1) similar to 1, clearly showing the existence of the nine-membered lactam ring. Four aromatic protons at δ 6.95 (1H, dd), 7.06 (1H, t), 7.28 (1H, dd) and 7.12 (1H, s) suggested that 2 was not substituted at positions 5, 6, 7 or 2. This spectrum is similar to that of 1, except for

![Fig. 1. Structures of (-)-14-O-Malonylindolactam-V (2), (-)-Indolactam-V (1) and Its 14-O-Acetate (3), and Teleocidin B-4.](image)

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the signals at position 14. A comparison between the spectra of 1 and 2 revealed that 2 was an ester at position 14. The acid moiety of the ester was found to be malonic acid from the 1H-NMR data (δ 3.38 (1H, d) and 3.42 (1H, d)) and 13C-NMR data (δ 42.11, 168.49, and 170.25). These signals were unambiguously assigned by the HMBC and HMQC spectra. The existence of the malonic acid residue was also confirmed by the IR spectrum [ν max (KBr) cm⁻¹: 1744 and 1717]. Alkaline treatment of 2 with 0.1 N KOH in methanol quantitatively produced 1 which was identical to an authentic sample by comparing the spectral data (δNH, 1H-NMR, and EIMS). Based on these data, 2 was determined to be (−)-14-O-malonyldolactam-V.

(−)-14-O-Malonyldolactam-V (2) is proposed to be a precursor of 1 and (−)-14-O-acetyldolactam-V (3) which have been isolated from S. blastmyceticae NA34-17. To confirm this hypothesis, 2 was subjected to heat treatment. Refluxing 2 in methanol mainly produced 1 (90%), along with a small amount of 3 (10%), indicating that 1 and 3 arose from 2 during the cultivation, concentration of the culture broth, and/or purification steps. It is especially noteworthy that the acetyl group of 3 was derived from the malonyl group after decarboxylation rather than from acetyl CoA. It is probable that some of the acetyl groups of other microbial metabolites are also derived from the corresponding malonic acid conjugates.

S. blastmyceticae NA34-17 is at present the sole microorganism to produce large quantities of 1 in the culture broth (30 mg/l). In normal teoilocidin-producing microorganisms, 1 cannot be isolated since it is not excreted from the cell and almost all of 1 is used for the teoilocidin biosynthesis. On the contrary, S. blastmyceticae NA34-17 might excrete 1 as a malonic acid conjugate to accumulate 1 in the culture broth after hydrolysis during cultivation. Although studies on malonic acid conjugates of microbial metabolites are rare, malonic acid conjugates might play an important role in the production of some secondary microbial metabolites.

References