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Anti-HIV Activity of Dehydroaltenusin- a Metabolite from a *Streptomyces* sp.

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Abstract – Dehydroaltenusin (**1**) was isolated from the chloroform extract of the culture filtrate of a *Streptomyces* sp. and its structure was determined from spectral data as well as by comparison with published values. In an XTT-based *in vitro* anti-HIV assay, dehydroaltenusin effectively inhibited the cytopathic effects of HIV infection at a concentration of 1-5 μg/mL.
Key words – Anti-HIV activity, dehydroaltenusin, *Streptomyces* sp.

Introduction

Streptomyces is widely known for the production of antibiotics (Waksman *et al.*, 1950; Dienstag and Neu, 1972; Argoudelis *et al,* 1987). As part of our continuing studies on the microbial metabolites from soil samples collected in different parts of Bangladesh (Jabbar *et al.*, 1994 and 1996), we recently reported the isolation, structure determination and antibacterial activity of dehydroaltenusinic acid from a *Streptomyces* sp. (Jabbar *et al.*, 1998). This paper describes the isolation and HIV-inhibitory activity of another compound, dehydroaltenusin (**1**) (Coombe *et al.*, 1970; Ayer and Racok, 1990; Fuska *et al.*, 1991; Proksa *et al.*, 1994) from the same organism. Although dehydroaltenusin (**1**), an inhibitor of growth and biochemical functions of *Ceratocystis pilifera, Aureobasidium pullulans* and *Cladosporium sphaerospermum* (Fuska *et al.*, 1991), has been previously reported from microorganisms of the genera *Alternaria* (Coombe *et al.*, 1970; Ayer and Racok, 1990) *Penicillium* (Proksa *et al.*, 1994) and *Talaromyces* (Fuska *et al.*, 1991), this is the first report of its occurrence from a *Sterptomyces* sp.

Experimental

General experimental procedure – Ultraviolet (UV) and infrared (IR) spectra were obtained on a Beckman DU-640 and Perkin-Elmer 1600 FTIR spectrometer, respectively. The ^{1}H-(500 MHz) and ^{13}C-(125 MHz) NMR spectra were recorded in $CDCl_3$ on a Varian VXR 500S spectrometer and the chemical shifts are reported in ppm relative to the residual non-deuterated solvents. The number of attached protons for ^{13}C signals was determined using the DEPT pulse sequence. Inverse detected heteronuclear correlations were measured using HSQC (optimized for $^1J_{CH}$ = 140 Hz) and HMBC (optimized for $^nJ_{CH}$ = 8.3 Hz) pulse sequences. Mass spectra and accurate mass measurements were performed on a VG Micromass ZAB mass spectrometer.

Collection of the *Streptomyces* sp. – The organism was collected from a soil sample in Dhaka, Bangladesh by the crowded plate technique (Hammond and Lambert, 1978) and it was identified as a *Streptomyces* sp. (Jabbar *et al.*, 1998).

Production, isolation and purification of dehydroaltenusin (1) – The strain of the *Streptomyces* sp. was grown in acidic Czapeks broth medium as described previously (Jabbar *et al.*, 1998) and the culture filtrate (975 ml) was extracted with $CHCl_3$ (100 ml × 5) at pH 2.5. Repeated preparative silica gel TLC (PF_{254}, 0.5 mm) of the crude extract using toluene-EtOAc-AcOH (24 : 5 : 1 drop) yielded dehydroaltenusin (**1**, 5.0 mg).

Dehydroaltenusin (1) – Yellowish gum; ^{1}H (500 MHz, $CDCl_3$): δ 11.30 (1H, s, OH-3), 6.73 (1H, d, J = 2.4 Hz, H-6), 6.67 (1H, s, H-7), 6.62 (1H, d, J = 2.4 Hz, H-4), 6.40 (1H, br s, OH-9), 6.28 (1H, s, H-10), 3.91 (3H, s, OMe-5), 1.73 (3H, s, Me-1a); ^{13}C (125 MHz, $CDCl_3$) δ 180.8 (C-8), 167.4 (C-2), 166.3 (C-5), 164.7 (C-3), 153.1 (C-7a), 146.1 (C-9), 135.0 (C-6a), 120.8 (C-7), 116. 2 (C-10), 104.4 (C-6), 103.6 (C-4), 99.8 (C-2a), 79.1 (C-1a), 56.0 (OMe), 29.5 (Me); HR-

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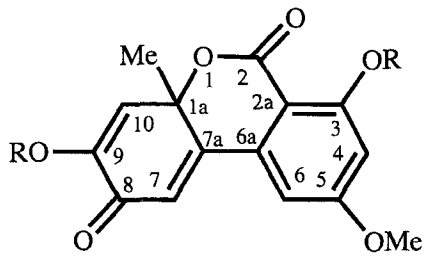

1 R = H
2 R = Ac

EIMS: *m/z* (rel. int.): 288.0654 (M^+, calcd for $C_{15}H_{12}O_6$ requires 288.0639, 100).

Acetylation of dehydroaltenusin (1) – Dehydroaltenusin (2.0 mg) was dissolved in 0.5 ml of pyridine and 1.0 ml of acetic anhydride was added. The reaction mixture was left at room temperature, in the dark for 48 hours. Evaporation of the reaction mixture under a stream of N_2 afforded virtually pure dehydroaltenusin diacetate (**2**).

Dehydroaltenusin diacetate (2) – Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.77 (1H, d, J = 2.4 Hz), 6.60 (1H, d, J = 2.4 Hz), 6.35 (1H, s), 6.30 (1H, s), 3.86 (3H, s, OMe), 2.42 (3H, s, OAc), 2.30 (3H, s, OAc), 1.72 (3H, s, Me); EIMS: *m/z* 372.0848 (M^+, calcd for $C_{19}H_{16}O_8$ requires 372.0845, 35).

Anti-HIV assay – Dehydroaltenusin was dissolved in DMSO, diluted to the desired concentration and tested in the U.S. National Cancer Institutes XTT-based anti-HIV assay, the experimental details of which have been published previously (Gulakowski *et al.*, 1991).

Results and Discussion

Dehydroaltenusin (**1**) was isolated from the chloroform extract of the culture filtrate of a *Streptomyces* sp. by repeated preparative TLC over silica gel. Its structure was independently deduced as dehydroaltenusin by spectral data interpretation and chemical conversion to its diacetate (**2**) and confirmed by comparison with previously reported values (Coombe *et al.*, 1970; Ayer and Racok, 1990; Fuska *et al.*, 1991; Proksa *et al.*, 1994). Dehydroaltenusin was tested for HIV-inhibitory properties as described elsewhere (Gulakowski *et al.*, 1991). In brief, the assay utilizes the RF strain of HIV-1 and the human lymphoblastic target cell line CEM-SS. Cytoprotection from virus-induced cell killing is measured by the metabolic reduction of the tetrazolium salt XTT to a colored formazon derivative by the surviving cells. The known anti-HIV drug, AZT is used as a positive control. Dehydroaltenusin effectively inhibited the cytopathic effects of HIV-1 infection at a concentration range of 1-5 μg/mL with maximum protection of 75-90% but was cytotoxic to the host cell at 6-8 μg/mL (Figure-1).

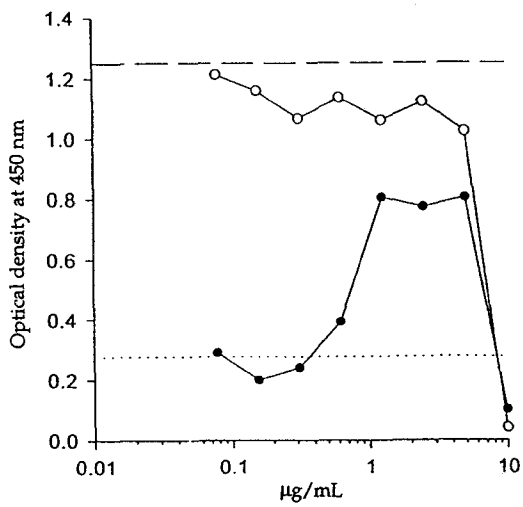

Fig. 1. Graph showing the effects of dehydroaltenusin upon uninfected CEM-SS (○) and HIV-1 infected CEM-SS cells (●), as determined after 6 days of culture. The higher optical density represents better anti-HIV activity exhibited by the test compound.

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