ISOLATION OF RP 71955, A NEW ANTI-HIV-1 PEPTIDE SECONDARY METABOLITE

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The genome of the human immuno deficiency virus (HIV-1) encodes a proteinase which processes the gag-pol and the gag polyproteins[1]. Inhibition of these processing steps results in the production of progeny virions which are immature and non infectious[2]. Thus the HIV-1 protease, which belongs to the aspartic proteinase family[3], has become an important target for the search of anti-HIV-1 compounds. In the course of our screening program to find inhibitors of this enzyme using a fluorometric assay[4] we have isolated a novel metabolite.

For the production of this compound the Streptomyces strain SP9440 was inoculated into a 250-ml Erlenmeyer flask containing 50 ml of seed medium consisting of corn steep 1%, sucrose 3%, calcium carbonate 7.5% and ammonium sulfate 2%. The seed culture was incubated for 3 days at 28°C on a rotary shaker (250 rpm). 5 to 12 ml of the culture was then transferred to a 2-liter Erlenmeyer flask containing 250 ml of the same medium and the culture was incubated for 3 other days at 28°C on a rotary shaker (150 rpm). The whole culture was transferred into a 100-liter fermentor containing 45 liters of the same medium and incubated at 28°C under agitation (400 rpm) and aeration (4 m^3/hour) for 2 days. 40 liters of the culture was then transferred into a 800-liter fermentor containing 400 liters of a medium consisting of yeast extract 1%, glucose 3%, calcium carbonate 5%, sodium chloride 2%, potassium dihydrogen phosphate 0.1% and magnesium sulfate 0.1%, and incubated at 28°C under agitation (150 rpm) and aeration (15 m^3/hour) for 192 hours. The broth (440 liters) was centrifuged and the supernatant was discarded. The mycelium was extracted with acetone (300 liters) and then centrifuged. The pellet was discarded. The acetone was removed under reduced pressure from the supernatant and the resulting aqueous phase (60 liters) was applied on a Duolite S 861 stainless steel column (20 × 60 cm). The column was washed with water (60 liters) and eluted with MeOH - H_2O (1 : 4) (60 liters), 50% MeOH (30 liters) and finally MeOH (60 liters) at a flow rate of 500 ml/minute. The methanolic fraction which contains the active metabolite was concentrated under reduced pressure to yield an aqueous phase (10 liters) which was applied on a Diaion HP20 (Mitsubishi Chemical Industries Limited) glass column (10 × 15 cm) to adsorb the metabolite. The column was washed with water and eluted with MeOH (8 liters). This fraction was made 50% MeOH and subjected to a preparative HPLC using an Amicon C18 (20 μm, 100 A) stainless steel column (7.62 × 50 cm) fitted with a stainless steel precolumn. The column was first washed with 50% MeOH (2 liters). A step gradient elution of increasing methanolic concentration from MeOH - H_2O (3 : 2) to MeOH - H_2O (4 : 1) was then performed and 800 ml fractions were collected at a flow rate of 200 ml/minute. The active eluate (MeOH - H_2O (7 : 3) and (4 : 1) fractions) was concentrated under reduced pressure to yield a crude powder (9.2 g). An aliquot (586 mg) was dissolved in a mixture of MeOH and DMSO 10 : 1 and chromatographed on a Sephadex LH20 column (3 × 150 cm) developed with MeOH. Flow rate was 1 ml/minute and 15 ml fractions were collected. The active fractions were collected and taken to dryness to yield 445 mg of a beige powder. An aliquot (223 mg) was dissolved in 60 ml of 0.1 N HCl - MeOH (1.1) and applied on a Amicon C18 (20 μm, 60 A) column (2.5 × 6 cm). The column was washed with 60 ml of MeOH - H_2O (5.5 : 4.5) and eluted with a step gradient of increasing methanolic concentration from MeOH - H_2O (6.5 : 3.5) to MeOH - H_2O (9 : 1). 5 ml fractions were collected at a flow rate of 10 ml/minute. The active eluate (MeOH - H_2O (4 : 1),

Fig. 1. Structure of RP 71955.

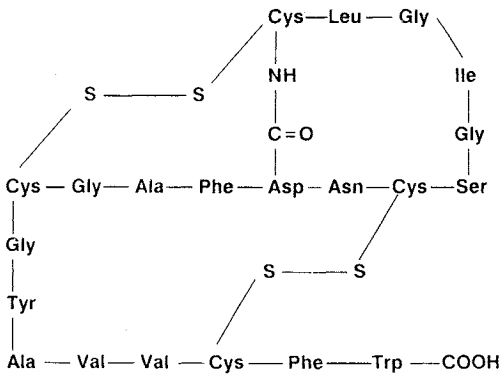

Table 1. Physico-chemical properties of RP 71955.

Appearance	White powder
$[\alpha]_D^{20}$	$-77°$ (c 0.1, MeOH)
SI-MS $(M+H)^+$	
Calcd for $C_{97}H_{132}O_{26}N_{23}S_4$	2,164.4
Found:	2,164.4
UV λ_{max}^{MeOH} nm (ε)	283 (6,200), 290 (4,700)
IR (KBr) cm^{-1}	3400, 3045, 2945, 1740 (sh), 1670, 1530, 1210, 875, 750, 600
Rf value[a]	0.45
Solubility	
Soluble	MeOH, DMSO
Insoluble	Hexane, $CHCl_3$, EtOAc

[a] Merck Art. No. 5715, ethyl acetate-acetic acid-water (4:1.2:1).

(8.5:1.5) and (9:1) fractions) was concentrated under reduced pressure to yield 143 mg of the pure metabolite as a colorless powder. The physico-chemical properties of the metabolite are summarized in Table 1. The structure of RP 71955 was assigned by NMR studies[5] and was shown to be an original peptidic secondary metabolite having its *N*-terminal amino acid acylated by the β carboxylic acid of the aspartic residue (Fig. 1). RP 71955 inhibited the HIV-1 aspartyl protease (IC_{50}, 35 μg/ml) and was found to be active in a T lymphocyte HIV-1 infection assay (A. BOUSSEAU, personal communication) as measured by the inhibition of the cytopathogenic effect of the virus (IC_{50}, 1 to 3 μg/ml) as well as by the inhibition of the production of reverse transcriptase (IC_{50}, 4 μg/ml).

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