

¹H-NMR Assignment of HIV Protease Inhibitor, Procyanidin B3 isolated from *Rosa rugosa*

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Abstract – The underground part of *Rosa rugosa* Thunb. has been used in Korean folk medicine for treating diabetes. The ¹H-NMR signal of procyanidin B3 isolated from *Rosa rugosa* was fully assigned by utilizing ¹H-¹H COSY. Procyanidin B3 showed a moderate inhibitory activity against HIV-1 protease.

Key words – *Rosa rugosa*, procyanidin B3, HIV-1 protease.

Introduction

The underground part of *Rosa rugosa* Thunb. (Rosaceae) has been used as antidiabetes in Korean folk medicine. Isolations of sterol, triterpenoid glycoside (Young *et al.*, 1987), flavonoids (Park and Ok, 1993a), polyphenolic compounds (Okuda *et al.*, 1982a; 1982b; Hatano *et al.*, 1990a; 1990b; Park *et al.*, 1993b), sesquiterpenoids (Hashidoko *et al.*, 1989; 1993; 1994), carotenoids (Razungles *et al.*, 1989), phenoxychromones (Hashidoko *et al.*, 1991), and alkyl esters (Hashidoko *et al.*, 1992) from *R. rugosa* were previously reported. In the present paper we report full assignment of ¹H-NMR signal and inhibitory effect of procyanidin B3 isolated from the underground part of *R. rugosa* against human immunodeficiency virus type 1 (HIV-1) protease.

Experimental

Plant material and apparatus – The underground part of *R. rugosa* was collected on July 10, 1992 in Hampyong, Jeonnam, Korea and a voucher specimen (No: NM 0351) is deposited in the Herbarium of Sunchon National University, Korea. NMR spectrum was recorded in CD₃OD+D₂O on a Varian VXR-500 spectrometer.

Isolation of compound 1 – The 1.5 kg of the air-dried underground part of *R. rugosa* was extracted with MeOH to give an MeOH extract (158 g), and this extract was partitioned into CH₂Cl₂ (36 g), EtOAc (28 g), *n*-BuOH (34

g), and water (37 g) fractions, respectively. The *n*-BuOH soluble fraction (25 g) was chromatographed over silica gel (5.7×45 cm) column with CHCl₃-MeOH-H₂O (65:35:10, lower phase) to yield impure 1 (37 mg) between 900 and 1050 mL. This sample was rechromatographed on Sephadex LH-20 (2.5×12 cm) with increasing concentrations of MeOH in H₂O (0% → 40% → 100% MeOH) to give compound 1 (28 mg) between 150 and 225 mL.

HPLC – Compound 1 was confirmed to be pure by normal and reversed HPLC analyses. HPLC was analysed under the following conditions. 1) normal phase; column: YMC-Pack SIL A-003; solvent: *n*-Hexane:MeOH:THF:HCOOH = 55:33:11:1 + (COOH)₂ 450 mg/L; flow rate: 1.5 mL/min; detection: UV 280 nm; detector: SPD-6A; pump: LC-6A; 2) reverse phase; column: YMC-Pack ODS A-302; solvent: 0.01 M H₃PO₄:0.01 M KH₂PO₄: EtOH:EtOAc = 47.5:47.5:3:2; flow rate: 1.0 mL/min; detection: UV 280 nm; detector: SPD-6A; pump: L-6000; temperature: 40°C.

Compound 1 – CD (MeOH); [θ]_{214.5} -1.6×10⁵, [θ]₂₃₆ -5.7×10⁴, [θ]_{272.5} 5.4×10²; ¹H-NMR (CD₃OD+D₂O, 500 MHz) see Table 1.

Assay of HIV-protease – The fused recombinant HIV-1 protease was prepared in Prof. Hattori's lab. as reported previously (Kusumoto *et al.*, 1995). A substrate, His-Lys-Ala-Arg-Val-Leu-(pNO₂-Phe)-Glu-Ala-Nle-Ser-NH₂, was purchased from Peptide Institute, Inc. (Osaka, Japan). For the assay of HIV-1 protease, the stock solution of the prepared enzyme was diluted. The compound was dissolved in dimethyl sulfoxide (10% in the reaction mixture). A reaction mixture (5 μL) composed of 1 μL of 50 mM NaOAc (pH 5.0), 1 μL of a substrate solution, 1 μL of compound and 2 μL of recombinant fused HIV-1 protease

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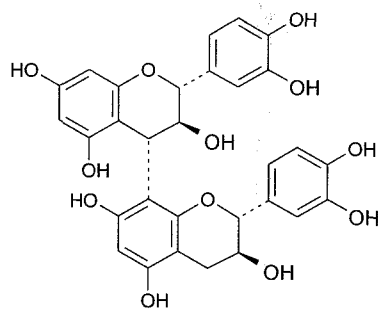
Table 1. ^1H NMR spectral data for procyanidin B3 from *Rosa rugosa* (500 MHz, in $\text{CD}_3\text{OD}+\text{D}_2\text{O}$)

H	Upper unit	Lower unit
H-2	4.45 (1H, d, $J = 8.0$ Hz)	4.58 (1H, d, $J = 7.5$ Hz)
H-3	4.39 (1H, dd, $J = 8.0, 10.0$ Hz)	3.83 (1H, dd, $J = 5.5, 7.5$ Hz)
H-4	4.29 (1H, d, $J = 10.0$ Hz)	2.53 (1H, dd, $J = 8.0, 16.0$ Hz)
H-6	5.83 (1H, d, $J = 2.5$ Hz)	2.80 (1H, dd, $J = 5.5, 16.0$ Hz)
H-8	5.93 (1H, d, $J = 2.5$ Hz)	6.11 (1H, s)
H-2'	6.63 (1H, d, $J = 2.0$ Hz)	6.78 (1H, d, $J = 2.0$ Hz)
H-5'	6.72 (1H, d, $J = 8.5$ Hz)	6.71 (1H, d, $J = 8.5$ Hz)
H-6'	6.29 (1H, dd, $J = 2.0, 8.5$ Hz)	6.50 (1H, dd, $J = 2.0, 8.5$ Hz)

solution was stirred, centrifuged and then incubated at 37°C for one hour in micro-tube. A control reaction was done under the same condition without compound. The reaction was stopped by heating at 90°C for one minute. $35\ \mu\text{L}$ of autoclaved water was added into the reaction mixture and an aliquot of $5\ \mu\text{L}$ was analyzed by HPLC. The hydrolysate and the remained substrate were quantitatively analyzed by HPLC under the following conditions: column, RP-C18 (150×4.6 mm i.d., YMC Co., Kyoto, Japan); elution, a linear gradient of CH_3CN (20~40%) in 0.1% TFA; injection volume, $5\ \mu\text{L}$; flow rate, $1.0\ \mu\text{L}/\text{min.}$; detection, 280 nm; system controller, Shimadzu SCL-6B; pump, Shimadzu LC-9A; detector, Shimadzu SPD-6A; recorder integrator, Shimadzu C-R 6A chromatopac; autoinjector, Shimadzu SIL-6B (all Shimadzu Co., Kyoto, Japan). The retention times of the substrate and Phe(NO_2)-bearing hydrolysate were 9.94 and 3.98 minutes, respectively.

Results and Discussion

Combination of column chromatographies using silica gel and Sephadex LH-20 of *n*-butanol fraction obtained from the methanol extract of the underground part of *R. rugosa* afforded compound **1**. The compound **1** is tentatively assigned to condensed tannin, procyanidin B3, which is a catechin-($4\alpha \rightarrow 8$)-catechin dimer, from J values of H-2 of upper and lower unit in ^1H NMR spectrum. CD spectrum

**Fig. 1.** Procyanidin B3 from *Rosa rugosa*.

supports this assignment. However, since the compound exists in a complex mixture of conformational isomers, it was difficult to assign unambiguously all the signals in NMR spectra and its upper and lower unit have not been clearly assigned in the literature. Even though it was difficult to distinguish the 1:1 ratio of conformers in acetone- d_6 , the major conformer of procyanidin B3 was assigned through ^1H - ^1H COSY in CD_3OD where one of conformers can exist predominantly compared to the other (Table 1). To the best of our knowledge this is the first report of full assignment of ^1H NMR spectrum of procyanidin B3. This compound had previously been isolated from *Sanguisorba officinalis* (Tanaka *et al.*, 1983), *Pyrola incarnata* (Yazaki *et al.*, 1989), *R. laevigata* (Yoshida *et al.*, 1989), *Geranium thunbergii* (Yazaki *et al.*, 1991), and *Cistus incanus* (Petereit *et al.*, 1991), however it was first found from the underground part of *R. rugosa*. Procyanidin B3 showed a moderate inhibitory activity against HIV-1 protease at a concentration of $100\ \mu\text{M}$ by $19.8\pm 2.3\%$ ($M\pm\text{SE}$, $n=3$). HIV possesses some enzymes that work on viral replication, such as RNA-dependent DNA polymerase or reverse transcriptase, RNA integrase, and protease. The maturation of the virus takes place at the last step by viral protease, which cleavage the viral polyproteins at the specific amino acid sequences to give functional proteins or enzymes. The mature viruses bud from the cells and continuously infect other T-cells. Recently, clarification on the structure and function of viral enzyme, a protease, has shown an important target for the design of antiviral agent for AIDS (Mcquade *et al.*, 1990; Meek *et al.*, 1989). Investigation of further anti-HIV protease compound is now in progress.

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