Inhibitory Constituents against HIV-1 Protease from *Agastache rugosa*

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Two diterpenoid compounds, agastanol (**1**) and agastaquinone (**2**), were isolated from the roots of *Agastache rugosa* (Labiatae). Compound **1** and **2** showed significant inhibitory effects against human immunodeficiency virus type 1 (HIV-1) protease activity with IC_{50} values of 360 and 87 μM, respectively.

Key words : *Agastache rugosa*, Labiatae, Agastanol, Agastaquinone, Anti-HIV-1 protease activity

INTRODUCTION

The cure and prevention of acquired immunodeficiency syndrome (AIDS) has been a global challenge since it was discovered, but nontoxic and less side effect anti-AIDS drugs are still in demand. Most of the developments for anti-AIDS drugs are based on blocking the steps of the viral life cycle, such as adsorption of the virus particle to the host cell, synthesis of viral DNA by reverse transcriptase, viral proteolytic process by protease and synthesis of viral envelope glycoproteins (Mitsuya *et al.*, 1987). The polyproteins are proteolytically processed by the action of a virus-encoded protease and created the functional proteins (Henderson *et al.*, 1988). Therefore, the inhibition of HIV-1 protease has been a promising target for the development of antiviral agents for AIDS (Ido *et al.*, 1991). In order to find HIV-1 protease inhibitory substances from natural products, we isolated several compounds from *Areca catechu,* (Kusumoto *et al.*, 1995), *Swietenia mahagoni* (Matsuse *et al.*, 1997) and *Ganoderma lucidum* (El-Mekkawy *et al.*, 1988; Min *et al.*, 1998). In the continuing study, we found that agastanol (**1**) and agastaquinone (**2**) from *Agastache rugosa* showed the inhibitory activities against HIV-1 protease.

MATERIALS AND METHODS

General experimental procedures

Melting points were obtained with an Electrothermal Series IA9100 apparatus and were not corrected. UV spectra were taken in *n*-hexane and MeOH on a Milton-Roy Spectronic 3000 spectrophotometer. IR spectra were recorded on a Precision Analect RFX-65 spectrophotometer. Mass spectra were obtained on a Kratos Concept-1S spectrometer at 70 eV. ^{1}H- and ^{13}C-NMR experiments were run in $CDCl_3$ containing TMS as the internal standard, using Varian Unity-300 and Bruker AM-500 spectrometers. Elemental analysis was carried out with a Carlo Erba EA1108 elemental analyzer.

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Plant material

The roots of *A. rugosa* were collected at Yangsan (Kyongnam Province, Korea) in October 1992. The voucher specimen are deposited in the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Korea.

Extraction and isolation

The air-dried roots (4.5 kg) of *A. rugosa* were ground and extracted with methanol (10 liters x 3) followed by a mixture of *n*-hexane-EtOAc-Me_2CO (4:4:2) (8 liters, 3×) at room temperature. The combined extracts were concentrated and extracted with *n*-hexane (500 ml, 4×). From *n*-hexane extract (50 g), agastanol (**1**, 160 mg) and agastaquinone (**2**, 150 mg) were isolated from repeated column chromatography and recrystallization method.

Agastanol (1): Pale yellow needle crystals (*n*-hexane). mp 180~182°C. $C_{21}H_{28}O_4$ (Found: C 72.88%, H 8.08, Calcd.: C 72.23%, H 8.17%). CIMS *m/z*: 345 (MH^+), EIMS *m/z*: 344 (M^+, base peak). UV λ_{max} (MeOH): 240, 279, 372. IR ν_{max} KBr (cm^{-1}): 3360, 2960, 1612. ^{1}H- and ^{13}C-NMR data: see reference (Lee *et al.*, 1994).

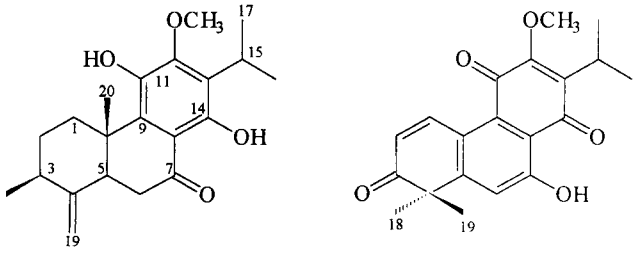
Agastaquinone (2): dark red needles, mp 121~122°, $C_{20}H_{20}O_5$ (Found: C 70.78%, H 6.05%, Calcd.: C 70.59 %, H 5.88%). UV λ_{max} (MeOH, log ε): 235 sh (3.91), 296 (3.93), 340 (3.51), 454 (3.46). IR ν_{max} KBr (cm^{-1}): 2973, 1668, 1658, 1630, 1610, 1292, 1275, 1259, EIMS *m/z* (rel. int.): 340 (M^+, 100). ^1H- and ^{13}C-NMR data: see reference (Lee *et al.*, 1995).

Protease assay

Twenty five μl of HIV-1 protease assay buffer (Bachem HIV protease assay Kit S-1000) containing 2.5 μg of a substrate, His-Lys-Ala-Arg-Val-Leu-(pNO_2-Phe)-Glu-Ala-NLe-Ser-NH_2, was mixed with 2.5 μl of a dimethyl sulfoxide (DMSO) solution of test compound, then 2.5 μl of recombinant HIV 1-protease (0.175 μg protein) was added to the mixture. After incubation at 37°C for 20 min, the reaction was stopped by addition of 2.5 μl of 10% TFA. The hydolysate and the remained substrate were quantitatively analyzed by HPLC under the following conditions: column, RP-C18 (150×4.6 mm i.d., YMC Co.); elution, a linear gradient of CH_3CN (20~40%) in 0.1% TFA; injection volume, 5 μl; flow rate, 1.0 ml/min; detection, 280 nm. The hydrolysate and substrate were eluted at 5.1 and 10.8 min, respectively. The inhibitory activity of the compound in the HIV-1 protease assay was calculated as follows: % inhibition= $100\times(A_{control}-A_{sample})/(A_{control})$; where A is a relative peak area of the hydrolysate. Acetyl pepstatin was used as a positive control with an IC_{50} of 0.24 μM under the above conditions.

RESULTS AND DISCUSSION

The whole plant of *A. rugosa* has been used as an agent for the treatment of cholera, vomiting, and miasma. It is considered useful in treating influenza or colds, headache, indigestion, fever, cholera and the nausea of pregnancy. In previous study, we isolated two new diterpenoid compounds, agastanol (**1**) and agastaquinone (**2**), from the roots of *A. rugosa*. Agastanol (**1**), pale yellow needles, mp 180~182°C, $C_{21}H_{28}O_4$, showed cytotoxic activities (ED_{50}: 4.9~29.9 ug/ml) against several human cancer cells (A549, SK-OV-3, SK-MEL-2, XF498 and HCT15). It showed weak antifungal activity against *Trichopyton rubrum* (Lee *et al.*, 1994). Agastaquinone (**2**), dark red needles, mp 121~122 °C, $C_{20}H_{20}O_5$, showed nonspecific cytotoxic activities (ED_{50}: 1.8~12.8 ug/ml) against same human cancer cell lines *in vitro* (Lee *et al.*, 1995). They were tested for the inhibitory activities against HIV-1 protease. The compound **1** showed weak inhibitory activity with IC_{50} value of 360 μM. While compound **2** showed significant inhibitory activity with IC_{50} value of 87 μM (Table II). Both case of cytotoxicity assay and HIV-1 protease assay, quinone compound, agastaquinone (**2**), showed higher cytotoxic effect against human cancer cell lines and inhibitory activity on HIV-1 protease than hydroxyl compound, agastanol (**1**). The active mechanism of quinone moiety in diterpene compound will be of particular interest to be investigated in future. In natural products, several diterpene compounds have been described as antiviral compounds. Prostatin, a phorbol ester type, showed potent anti-HIV activity without tumor-promoting effect (Gustafson *et al.*, 1992). Tripterifordin and neotripterifordin from *Tripterygium wilfordii* inhibited HIV-1 in H9 lymphocyte cells (Chen *et al.*, 1992, 1995). 6-Hydroxytremetone from *Werneria cilliolata* showed a significant inhibition of HIV-1 replication (Piacente *et al.*, 1994). Based on the inhibitory activities against HIV-1 protease from natural products, more detailed study will be needed to clarify the action of diterpenoid compounds from *A. rugosa*.



Agastanol (**1**) Agastaquinone (**2**)

Fig. 1. Structures of agastanol (**1**) and agastaquinone (**2**)

Table I. HIV-1 protease inhibitory activities of compound **1** and **2**

Compound	IC_{50} (μM)
Agastanol (**1**)	360
Agastaquinone (**2**)	87
Acetyl pepstatin[a)]	0.24

[a]Positive control

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