Testosterone 5 α -Reductase Inhibitory Active Constituents from *Anemarrhenae Rhizoma*

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The diethyl ether extract of *Anemarrhenae Rhizoma* (rhizomes of *Anemarrhena asphodeloides* BUNGE) showed testosterone 5 α -reductase inhibitory activity. Two major constituents, *cis*-hinokiresinol (1**) and 2,6,4'-tri-hydroxy-4-methoxybenzophenone (**2**) were identified as the active principles. The inhibitory activity of **1** was superior to that of ethinylestradiol, but that of **2** was weak.**

Key words *Anemarrhenae Rhizoma*; *Anemarrhena asphodeloides*; testosterone 5 α -reductase inhibitor

Testosterone 5 α -reductase catalyzes the conversion of testosterone to an active androgen, dihydrotestosterone, which binds to androgen receptors and shows various hormonal actions. An excessive accumulation of dihydrotestosterone is recognized as leading to male pattern baldness and benign prostatic hyperplasia.^{1,2)} Therefore, treatment with a testosterone 5 α -reductase inhibitor would be expected to lead to a decrease of dihydrotestosterone concentration in many tissues, and may be useful for protection of depilation. During the course of our search for natural crude drugs having testosterone 5 α -reductase inhibitory activity,³⁾ the diethyl ether extract of *Anemarrhenae Rhizoma* (rhizomes of *Anemarrhena asphodeloides* BUNGE, Liliaceae) showed a remarkable activity (inhibition %: concentration of the diethyl ether extract in 50% ethanol; 77.4%: 0.5 mg/ml, 91.9%: 2 mg/ml). *Anemarrhenae Rhizoma*, prescribed as antipyretic, sedative, and diuretic in Chinese medicine, has been shown to contain several kinds of compounds, e.g., saponins having PAF-induced platelet aggregation inhibitory activity,⁴⁾ xanthones, and phenols exhibiting cyclic AMP phosphodiesterase inhibitory activity;⁵⁾ however, no testosterone 5 α -reductase inhibitory effect has been reported. Activity guided chromatography of the active extract led to the isolation of two active compounds, *cis*-hinokiresinol (**1**)⁵⁾ (IC₅₀, 0.31 mM) and 2,6,4'-trihydroxy-4-methoxybenzophenone (**2**)⁶⁾ (IC₅₀, >1.00 mM) (Table 1). The inhibitory activity of **1** was superior to that of ethinylestradiol (IC₅₀, 0.81 mM), but that of **2** was weak. This finding implies that the extract of *Anemarrhenae Rhizoma* may be useful for hair-care products depending on its testosterone 5 α -reductase inhibitory activity. Compounds **1** and **2** have been isolated from *Anemarrhena asphodeloides*.⁵⁾ Although **1** has an alternative name, nyasol⁷⁾ which was obtained as an aglycon of nyasoside isolated from *Hypoxis nyasica*, we used here the name of *cis*-hinokiresinol whose absolute configuration has not been determined. Using the diethyl ether extract of *Anemarrhenae Rhizoma* and **1**, several *in vivo* tests as a hair growth promoter are in progress.

MATERIALS AND METHODS

Testosterone 5 α -reductase was prepared according to the method of Imai.⁸⁾ Twenty-four hour-fasted rat (male, Slc-SD strain, aged 6 weeks) liver was isolated after circulation with

ice-cold Krebs-Ringer phosphate buffer (pH 7.4). The isolated liver was homogenized with 5 volumes of ice-cold Tris-HCl buffer (10 mM, pH 7.2) and centrifuged at 9000×*g* (10 min). The supernatant was stored at –80 °C until use. 5 α -Reductase assay was performed according to the method described by Iбата⁹⁾ with minor modification. The reaction solution contained 50% ethanol solution (0.2 ml) of various concentrations of test compounds, 1.0 ml of Tris-HCl buffer pH 7.2, 0.3 ml of testosterone (500 μ g/ml in propylene glycol-Tris-HCl buffer pH 7.2 (1 : 1 v/v)), and 1.0 ml of the enzyme solution. The reactions were started by the addition of 0.5 ml of NADPH (0.77 mg/ml in Tris-HCl buffer pH 7.2). The mixture was incubated at 37 °C for 30 min, and the reaction was stopped by the addition of 5.0 ml of dichloromethane. After addition of 0.5 ml of hexyl *p*-hydroxybenzoate (0.1 mg/ml in methanol, an internal standard (I.S.) for HPLC), the tube was shaken for 10 min and centrifuged at 900×*g* (10 min). The organic layer (4 ml) was transferred to another tube and evaporated to dryness. The residue was dissolved in 5.0 ml of methanol, and an aliquot of 10 μ l was injected into the HPLC system (Jasco 880-PU, 875-UV). HPLC conditions: column; YMC-Pak ODS-AM302 (150×4.6 mm I.D.) at 40 °C, mobile phase; methanol-H₂O (65 : 35 v/v), flow rate; 1.0 ml/min, detection; UV 254 nm, *t*_R; testos-

Table 1. Inhibitory Effects of Compounds **1**, **2** and Ethinylestradiol on *in Vitro* Testosterone 5 α -reductase Activity

Compound	Concentration (mM)	Inhibition ^{a)} (%)	IC ₅₀ (mM)
1	0.01	2.4±0.4	0.31
	0.05	7.6±0.7	
	0.2	40.9±1.6	
	0.5	77.0±1.6
2	1.0	14.0±5.8	>1.00
Ethinylestradiol	0.01	8.5±2.0	0.81
	0.05	23.3±0.7	
	0.2	37.9±1.3	
	1.0	55.1±2.9	

a) Each value represents the mean±S.E. of 3 experiments.

terone, 7 min, I.S., 14 min. Control-0 min tube received 5.0 ml of dichloromethane before addition of NADPH, while control-30 min tube received 0.2 ml of 50% ethanol instead of test sample. A similar procedure to that described above was carried out for these control tubes. 5 α -Reductase inhibitory activity was determined from the following equation using the peak-area ratios (r =testosterone/I.S.).

$$\text{inhibition (\%)} = 100 \times (r \text{ of test sample} - r \text{ of control} - 30 \text{ min}) / (r \text{ of control} - 0 \text{ min} - r \text{ of control} - 30 \text{ min}).$$

Anemarrhenae Rhizoma (dried rhizomes of *Anemarrhena asphodeloides* BUNGE produced in China) was purchased from Yamada Yakken Co., Ltd. Pharmacy (Osaka, Japan). A voucher specimen has been deposited at Kinki University, Osaka (voucher number: ASP-97001). The crushed rhizomes (5 kg) were extracted three times successively with diethyl ether (10 l each) to give 36 g of an extract as brown powder. The extract (31 g) was chromatographed over silica gel (Merck, No. 1.07734) eluted with a gradient of benzene and ethyl acetate to give the following four fractions: fraction 1 (benzene 100% elute, yield; 4.9 g, inhibition %; 2.7% at 0.5 mg/ml), fraction 2 (benzene–ethyl acetate (10:1) elute, yield; 11.4 g, inhibition %; 82.8% at 0.5 mg/ml), fraction 3 (benzene–ethyl acetate (7:1) elute, yield; 3.3 g, inhibition %; 83.6% at 0.5 mg/ml), fraction 4 (benzene–ethyl acetate (5:1) elute, yield; 4.9 g, inhibition %; 75.1% at 0.5 mg/ml). Repeatedly activity-guided chromatography of fraction 2 (benzene–ethyl acetate (10:1) elute) over silica gel eluted with chloroform afforded 46 mg of **1** as a colorless oil. Recrystallization of fraction 4 (benzene–ethyl acetate (5:1) elute) from ethyl acetate–*n*-hexane gave 359 mg of **2** as yellow prisms mp 176–178 °C. The structures of **1** and **2** were deduced from the comparison of their mass and NMR spectral data with those of the reported data.^{6,7)}

The biological experimental data were tested for statistical significance by means of Bonferroni/Dunn's multiple range test method.

cis-Hinokiresinol (**1**; Nyasol, Z-1,3-Bis-4'-hydroxyphenyl-

1,4-pentadiene): [α]_D²⁰ –51°, (c =3.0, CHCl₃). C₁₇H₁₆O₂, EI-MS: m/z (% relative intensity) 252 (M⁺, 100), 237 (32), 158 (67), 145 (65), 131 (49), 107 (92). ¹H-NMR (500 MHz, CDCl₃) δ : 4.49 (1H, bdd, J =10.1, 6.2, H-3), 4.72, 4.83 (1H each, br s, 4'-OH, 4''-OH), 5.16 (1H, dd, J =9.5, 1.5, H-5E), 5.17 (1H, dd, J =17.6, 1.5, H-5Z), 5.68 (1H, dd, J =11.4, 10.1, H-2), 6.01 (1H, ddd, J =17.6, 10.1, 6.2, H-4), 6.52 (1H, d, J =11.4, H-1), 6.77 (2H, d, J =8.2, H-3', 5'), 6.79 (2H, d, J =8.6, H-3', 5'), 7.10 (2H, d, J =8.2, H-2'', 6''), 7.17 (2H, d, J =8.6, H-2', 6'); ¹³C-NMR (125 MHz, CDCl₃) δ : 30.0 (C-1'), 129.8 (C-2', 6'), 115.1 (C-3', 5'), 154.5^a (C-4'), 128.8 (C-1), 131.7 (C-2), 46.8 (C-3), 140.7 (C-4), 115.0 (C-5), 135.6 (C-1''), 128.9 (C-2'', 6''), 115.4 (C-3'', 5''), 154.0^a (C-4''), ^a interchangeable.

2,6,4'-Trihydroxy-4-methoxybenzophenone (**2**): C₁₄H₁₂O₅, EI-MS: m/z (% relative intensity) 260 (M⁺, 79), 259 (100), 167 (68), 166 (83), 138 (40), 121 (36), 93 (22). ¹H-NMR (500 MHz, acetone-*d*₆) δ : 3.80 (3H, s, OMe), 6.04 (2H, s, H-3, 5), 6.86 (2H, d, J =8.9, H-3', 5'), 7.63 (2H, d, J =8.9, H-2', 6'), 9.28 (1H, br s, 4'-OH), 10.06 (2H, br s, 2, 6-OH); ¹³C-NMR (125 MHz, acetone-*d*₆) δ : 106.4 (C-1), 162.5 (C-2, 6), 94.5 (C-3, 5), 166.1 (C-4), 197.8 (C=O), 133.1 (C-1'), 115.2 (C-2', 6'), 132.4 (C-3', 5'), 162.0 (C-4').

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