Natural Medicines **50**(6), 408-412 (1996)

Anti-Androgen Active Constituents from *Dalbergia cochinchinensis* PIERRE

MASANORI KUROYANAGI,*,[a] AKIRA UENO,[a] YUTAKA HIRAYAMA,[b] YUSUKE HAKAMATA,[b] TOMOO GOKITA,[b] TAKAKO ISHIMARU,[b] SYOJI KAMEYAMA,[b] TAKUMA YANAGAWA,[b] MOTOYOSHI SATAKE[c] and SETSUKO SEKITA[c]

[a] *School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yata, Shizuoka 422, Japan*
[b] *Lion Corporation, Biological Science Research Center, 100, Tajima, Odawara 256, Japan*
[c] *National Institutes of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158, Japan*

(Received May 7, 1996)

A methanol extract of *Dalbergia cochinchinensis* PIERRE showed a potent anti-androgen activity. From the extract, 10 constituents, **1**-**10** were isolated as its active constituents. Of these, 8, **9** and **10** were new compounds and their structures were elucidated by means of spectral methods. Of the 10 compounds, the main constituents, **1** and **2**, showed a potent anti-androgen activity: **2** had a potent inhibitory activity on testosterone 5α-reductase and **1** and **2** inhibited the binding of dihydrotestosterone to its receptor in a competitive mode.

Keywords——*Dalbergia cochinchinensis* PIERRE; Leguminosae; testosterone 5α-reductase; DHT receptor; neoflavone; anti-androgen activity

Testosterone, one of the androgens, is converted to 5α-dihydrotestosterone (DHT) by testosterone 5α-reductase[1] in the prostate gland or subaceous gland. The reduced product, DHT, is bound to its receptor and causes several biological effects.[2] Recently, it was reported that overproduction of DHT might be one of factors which cause male pattern bladness,[3] bening prostatic hyperplasia etc.[4] To obtain anti-androgen constituents from plant sources, which can be used for prevention and treatment of the diseases caused by over production of DHT, MeOH extracts of many kinds of samples were assayed. For the isolation and assay of anti-androgen substances from plant sources, two biological tests, i.e., determination of inhibitory activities on testosterone 5α-reductase and of the binding of DHT to its receptor, were carried out. Of these tested samples, a MeOH extract of *Dalbergia cochinchinensis* PIERRE showed potent activities in both tests.

D. cochinchinensis (Leguminosae) plants are cultivated in south-east Asia and used as high quality wood for building in our country.

This paper deals with the isolation and structural elucidation of the anti-androgen constituents from a MeOH extract of *D. cochinchinensis* and the activities of the isolated constituents.

MATERIALS AND METHODS

General experimental procedures Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were taken with JEOL GSX-500 and GSX-270 FT-NMR spectrometers, and chemical shifts were given in ppm with tetramethyl silane as an internal standard. FAB-MS were recorded on a JEOL JMX-SX102 mass spectrometer using *m*-nitrobenzylalcohol as a matrix, optical rotations with a JASCO DIP-360 digital polarimeter at 24℃, CD spectra with a JASCO J-20A spectropolarimeter, and UV spectra on a Hitachi U-3410 spectrophotometer. Kieselgel 60 (Merck) was used for column chromatography. Analytical and preparative HPLC was carried out on YMC R-ODS-7 packed columns.

Plant material The heart wood of *D. cochinchinensis* was purchased in Japan in 1992, which was botanically identified by Dr. Ken Ogata, Director of The Forestry and Forest Products Research Institute, Tohoku Research Center.

Extraction and isolation Chipped heart wood (150 g) of *Dalbergia cochinchinensis* was extracted with hot MeOH. The solvent was removed and the MeOH extract, showing a potent anti-androgen activity, was partitioned between EtOAc and H_2O. The EtOAc soluble fraction, showing the activity, was chromatographed on a silica gel column to give 13 fraction. Fr. 2 was recrystallized from hexane-EtOAc to give methoxydalbergion (**2**) (200 mg). Fr. 4 was purified by HPLC to give 5-*O*-methyllatifolin (**3**) (300 mg) and 2, 5-dihydroxy-4-methoxybenzophenone (**4**) (15 mg). Fr. 6 was recrystallized from hexane to give latifolin (**1**) (3 g). Frs. 8, 9, and 10 were purified by HPLC to give isoliquilitigenin (**5**) (200 mg), liquilitigenin (**6**) (250 mg) and calycosin (**7**) (25 mg), respectively. Fr. 12 was purified by HPLC to give compounds 8 (30 mg), **9** (15 mg) and **10** (40 mg).

Latifolin (1) Colorless needles, mp 119-120℃ (hexane-EtOAc), FAB-MS; *m/z* 286 $[M]^+$ $C_{17}H_{18}O_4$, $[\alpha]_D$ −23.1° (*c* = 2.9, MeOH).

Methoxydalbergion (2) Pale yellow needles, mp

102-104℃ (MeOH), FAB-MS; m/z 255 $[MH]^+$ $C_{16}H_{17}O_3$, $[\alpha]_D$ −2.8° (c=0.59, MeOH).

5-*O*-Methoxylatifolin (3) Viscous oil, FAB-MS; m/z 300 $[M]^+$ $C_{18}H_{20}O_3$, $[\alpha]_D$ −29.1° (c=0.74, Me).

2,5-Dihydroxy-4-methoxybenzophenone (4) Amorphous solid, FAB-MS; m/z $[MH]^+$ $C_{14}H_{13}O_4$, ^{1}H-NMR ($CDCl_3$); 3.96 (3H, s, OMe), 6.55 (1H, s, H-3), 7.10 (1H, s, H-6), 7.48 (2H, t, J=7.3 Hz, H-10, 12), 7.56 (1H, tt, J=7.0, 1.6 Hz, H-11), 7.63 (2H, dd, J=7.3, 1.4 Hz, H-9, 13), 12.5 (1H, s, chelated OH). The ^{13}C-NMR data are shown in TABLE I.

Isoliquilitigenin (5) Amorphous solid, FAB-MS; m/z 257 $[MH]^+$ $C_{15}H_{13}O_4$.

Liquilitigenin (6) Colorless needles, mp 207-208°C (MeOH), FAB-MS; m/z 257 $[MH]^+$ $C_{15}H_{13}O_4$, ^{1}H-NMR ($CDCl_3$); 2.63 (1H, dd, J=16.5, 2.5 Hz, H-3), 2.93 (1H, dd, J=16.5, 13.0 Hz, H-3), 5.22 (1H, dd, J=13.0, 2.5 Hz, H-2), 6.35 (1H, s, H-8), 6.78 (2H, d, J=8.5 Hz, H-3′, 5′), 7.18 (1H, s, II-5), 7.21 (2H, d, J=8.5 Hz, H-2′, 6′). The ^{13}C-NMR data are shown in TABLE II.

Calycosin (7) Amorphous solid, FAB-MS; m/z 285 $[MH]^+$ $C_{16}H_{13}O_5$.

Darbergiol (8) Amorphous solid, FAB-MS; m/z 302 $[M]^+$ $C_{17}H_{18}O_5$, ^{1}H-NMR ($CDCl_3$); 3.49 (3H, s, OMe), 3.76 (3H, s, OMe), 3.94 (2H, d, J=6.9 Hz, H-3′), 6.05 (1H, t, J=6.8 Hz, H-2′), 6.38 (1H, s, H-3), 6.65 (1H, dd, J=8.4, 1.1 Hz, H-3″), 6.67 (1H, s, H-6), 6.72 (1H, dt, J=7.3, 1.4 Hz, H-5″), 6.84 (1H, dd, J=7.3, 1.4 Hz, H-6″), 7.04 (1H, dt, J=8.1, 1.4 Hz, H-4″). The ^{13}C-NMR data are shown in TABLE I.

Compound 9 (9) Colorless powders, mp 219-223°C (MeOH), FAB-MS; m/z 287 $[MH]^+$ $C_{16}H_{15}O_5$. UV λ_{max}^{MeOH} ε (nm); 6,400 (345), 13,500 (275), 22,900 (238). ^{1}H-NMR ($CDCl_3$); 2.54 (1H, dd, J=16.5, 2.7 Hz, H-3), 2.86 (1H, dd, J=16.5, 13.2 Hz, H-3), 5.15 (1H, dd, J=13.2, 2.7 Hz, H-2), 6.30 (1H, s, H-8), 6.70 (2H, d, J=8.6 Hz, H-3′, 5′), 7.08 (1H, s, H-5), 7.15 (2H, d, J=8.6 Hz, H-2′, 6′). The ^{13}C-NMR data are shown in TABLE II. CD; $[\theta]_{348}$ +4.140, $[\theta]_{313}$ −7,100.

Compound 10 (10) Colorless needles, mp 237-239℃ (MeOH). FAB-MS; m/z 273 $[MH]^+$ $C_{15}H_{13}O_5$. UV λ_{max}^{MeOH} ε (nm); 5,800 (335), 10,400 (278), 16,100 (240). ^{1}H-NMR ($CDCl_3$); 2.64 (1H, dd, J=17.0, 2.5 Hz, H-3), 2.95 (1H, dd, J=17.0, 13.0 Hz, H-3), 5.22 (1H, dd, J=13.0, 2.7 Hz, H-2), 6.39 (1H, s, H-8), 6.78 (2H, d, J=8.5 Hz, II-3′, 5′), 7.18 (1H, s, H-5), 7.21 (2H, d, J=8.5 Hz, H-2′, 6′). The ^{13}C-NMR data are shown in TABLE II. CD; $[\theta]_{350}$ +6,360, $[\theta]_{315}$ −9,140.

Acetylation of 9 Thirty milligrams of **9** was acetylated with Ac_2O-pyridine at room temperature, overnight. The reaction solution was poured into ice-water and extracted with EtOAc. The organic solution was washed with dil-HCl, saturated $NaHCO_3$ solution and water, successively. The solution was dried to give a pale yellow product, which was recrystallized from MeOH to give colorless needles (**9a**), 25 mg. **9a**; mp 159-160℃. FAB-MS; m/z 299 $[MH]^+$ $C_{21}H_{19}O_8$, 357 $[MH-CH_2CO]^+$ $C_{19}H_{17}O_7$. ^{1}H-NMR ($CDCl_3$); 2.29, 2.30, 2.32 (3H each, s, Ac), 2.88 (1H, dd, J=16.4, 3.2 Hz, H-3), 3.04 (1H, d, J=16.4, 13.6 Hz, H-3), 5.49 (1H, dd, J=13.6, 3.2 Hz, H-2), 6.95 (1H, s, H-8), 7.16 (2H, d, J=8.4 Hz, H-3′, 5′), 7.48 (2H, d, J=8.4 Hz, H-2′, 6′), 7.72 (1H, s, H-5). ^{13}C-NMR ($CDCl_3$); 20.5, 20.7, 21.2 (Ac), 44.2 (C-3), 79.6 (C-2), 113.2 (C-8), 118.8 (C-10), 121.3 (C-5), 122.1 (C-3′, 5′), 127.4 (C-2′, 6′), 135.8 (C-1′), 37.1 (C-6), 148.4 (C-7), 151.0 (C-4′), 159.6 (C-9), 167.4, 168.4, 169.3 (CH_3COO), 190.0 (C-4).

Acetylation of 10 Thirty milligrams of **10** was acetylated by the same way as for **9** to give diacetate as colorless needles (**10a**), 20 mg. **10a**; mp 186-188℃. FAB-MS; m/z 371 $[MH]^+$ $C_{20}H_{19}O_7$, 329 $[MH-CH_2CO]^+$ $C_{18}H_{17}O_6$. ^{1}H-NMR ($CDCl_3$); 2.30, 2.31 (3H each, s, Ac), 2.82 (1H, d, J=16.8, 2.8 Hz, H-3), 3.0 (1H, dd, J=16.8, 13.2 Hz, H-3), 3.85 (3H, s, OMe), 5.47 (1H, dd, J=13.6, 2.8 Hz, H-2), 6.56 (1H, s, H-8), 7.16 (2H, d, J=8.4 Hz, H-3′, 5′), 7.48 (2H, d, J=8.4 Hz, H-2′, 6′), 7.58 (1H, s, H-5). ^{13}C-NMR ($CDCl_3$); 20.5, 21.2 (CH_3CO), 44.1 (C-3), 56.3 (OMe), 79.7 (C-2), 100.9 (C-8), 113.8 (C-10), 120.4 (C-5), 122.1 (C-3′, 5′), 127.4 (C-2′, 6′), 135.1 (C-6), 136.1 (C-1′), 150.9 (C-4′), 157.9 (C-7), 161.2 (C-9), 169.1, 169.3 (CH_3CO), 189.8 (C-4).

Assay for 5α-reductase

Preparation of enzyme solution Male Wistar rats, 10 to 12 weeks old, were sacrificated. The ventral prostates were immediately isolated and rinsed with cold (0-4℃) 50 mM Tris-HCl buffer, pH 7.4, containing 1.5 mM EDTA, 1 mM DTT, 10 mM Na_2MoO_4 and 10% glycerol (buffer A). The ventral prostates were then blotted with filter paper, weighed and homogenized with 5 volumes of the buffer A by using a Potter-Elvehjem teflon homogenizer under cooling with ice-water. The homogenate was centrifuged at 800×g for 10 min, and the supernatant was used as an enzyme solution.

Enzymatic assay A reaction mixture consisting of 0.25 ml of the enzyme solution, 0.02 ml of a [^{3}H]-testosterone ethanolic solution (100 mCi/ml, 70 Ci/mmol), 0.1 ml of buffer A containing 5 mM NADPH, 0.01 ml of an ethanolic solution of test sample, and 0.15 ml of buffer A was incubated for 60 min at 37℃. The reaction was terminated by the addition of 2 ml of Folch solution (chloroform-MeOH =2 : 1). Then, the reaction mixture was shaken for 30 s and centrifuging at 3,000 rpm for 10 min. The lower layer was separated and dried under an N_2 atmosphere at 80℃, and the residue was dissolved in 0.05 ml of MeOH, which was separated into three bands (testosterone, dihydrotestosterone, androstandiol) by TLC (Kieselgel 60F_{254}, Merck, chloroform-MeOH =100 : 1). The radioactivity of each band was measured by a radiochromanyzer (Aloka). The inhibitory activity (I.A.) of a test sample was calculated as follows:

Metabolic testosterone (M, %)
$= (D+A)/(T+D+A) \times 100$

T; counts of testosterone, D; counts of 5α-dihydro-

testosterone, A; counts of androstadiol.

Inhibitory activity (I.A., %) $= (1-M_s/M_c)\times 100$

M_s; M in the presence of test sample, M_c; M in the absence of test sample.

Assay for DHT reductase Male Wistar rats, 10 to 12 weeks old, were castrated under ether anesthesia. One day after castration, the animals were sacrificed. The vental prostates were immediately isolated, rinsed with cold buffer A, blotted with filter paper, weighed and homogenized with 4 volumes of buffer A by using a Potter-Elvehjem teflon homogenizer under cooling with ice-water. The homogenate was ultracentrifuged at $10,500\times g$ for 60 min at 4℃. The receptor solution (cytosol fraction) was obtained as the supernatant fluid.

Receptor assay A reaction mixture consisting of 0.1 ml of the receptor solution, 0.005 ml of 1 mM [^{3}H]DHT ethanolic solution, 0.005 ml of ethanolic solution of the test sample and 0.15 ml of buffer A was incubated for 16 h at 4℃. After incubation, free and the receptor complex form [^{3}H]DHTs were separated by addition of 0.25 ml of dextran-coated charcoal suspension containing 2.5% (w/v) charcoal and 0.5% (w/v) dextran in buffer A. After standing for 10 min at 4℃, the mixture was centrifuged at $800\times g$ for 10 min at 4℃. The radioactivity of the supernatant was measured by the liquid scintillation counter, the result of which was the total binding. Non-specific binding was estimated from the results of an analogous incubation mixture containing [^{3}H]DHT mixed with 400-fold excess of non-radioactive DHT. Specific binding was defined as the difference between the total binding and the non-specific binding. The mode of inhibitory action of test samples on receptor binding of DHT was analyzed by the method of double reciprocal plot of Lineweaver-Burk. The protein contents in tissue preparations were determined by the method of Lowry *et al.*

The inhibitory activity (I.A.) on the formation of DHT receptor complex was calculated as follows:

Inhibitory activity (I.A.) $= (1-B_{sample}/B_{control})\times 100$

B; the specific [^{3}H]DHT binding (fmol/ml protein).

RESULTS AND DISCUSSION

Since the EtOAc soluble fraction of a hot MeOH extract of heart wood of *D. cochinchinensis* showed a potent anti-androgen activity, the EtOAc soluble fraction was further purified by repeated silica gel column chromatography and HPLC using ODS column to give 10 compounds, **1**–**10**, of which **8**, **9** and **10** were new compounds. **1**–**3** were examined by FAB-MS, ^{1}H-NMR and ^{13}C-NMR spectra and identified as latifolin,[5,6] methoxydalbergion[7] and 5-*O*-methyllatifolin,[8] respectively. **4** was identified as 2,5-dihydro-4-methoxybenzophenone[9] from the spectral data. **5**–**7** were identified from the spectral data as isoliquilitigenin,[10] liquilitigenin[10] and calycosin,[6] respectively.

8 had the molecular formular, $C_{17}H_{18}O_5$, from the FAB-MS; m/z 302 [M]$^+$. The ^{1}H-NMR spectrum of **8** showed the presence of 2-hydroxyphenyl moiety [δ 6.65 (1H, dd, J=8.4, 1.1 Hz), 6.72 (1H, dt, J=7.3, 1.4 Hz), 6.84 (1H, dd, J=7.3, 1.4 Hz), 7.04 (1H, dt, J=1.4, 8.1 Hz)], 2,4-dimethoxy-5-hydroxyphenyl moiety [δ 3.49 (3H, s), 3.76 (3H, s), 6.38 (1H, s), 6.57 (1H, s)] as in the spectrum of **1**, hydroxymethyl group [δ 3.94 (2H, d, J=6.8 Hz)] and olefinic proton [δ 6.05 (1H, t, J=6.8 Hz)]. The ^{13}C-NMR spectrum of **8** showed presence of 2-hydroxyphenyl, 2,4-dimethoxy-5-hydroxyphenyl moieties as in the case of **1** (see in TABLE I), hydroxymethyl group (δ 60.1) and trisubstituted olefin moiety (δ 135.9, 131.9). From these data, the structure of **8** was elucidated to be a neoflavone derivative. The geometrical isomerism of olefin moiety was established from a difference nuclear Overhauser effect (NOE) experiment. The irradiation at H-6 induced its correlation with the olefinic H and the irradiation at the methylene proton induced its correlation with H-6″ as shown in Fig. 1. From these facts, **8** was determined to be 1-(2,4-dimethoxy-5-hydroxyphenyl)-1-(2-hydroxyphenyl)-1-pentene-3-ol and named darbergiol.

9 gave the molecular formula, $C_{15}H_{12}O_5$, from the FAB-MS; m/z 273 [MH]$^+$. Acetylation of **9** gave triacetate (**9a**), $C_{21}H_{18}O_8$. The ^{1}H-NMR spectrum of **9** showed the presence of the methine group [δ 5.15 (1H, dd, J= 13.2, 2.7 Hz)], methylene group [δ 2.54 (1H, dd, J = 16.5, 2.7 Hz), 2.86 (1H, dd, J =16.5, 13.2 Hz)] characteristic of a flavanone skeleton, two singlet aromatic protons [δ 6.30 (1H, s), 7.08 (1H, s)] and AA′BB′ type signals of aromatic ring [δ 7.15 (2H, d, J =8.6 Hz), 6.70 (2H, d, J =8.6 Hz)] supposed to be caused by *p*-hydroxyphenyl moiety. The ^{13}C-NMR spectrum of **9** also showed the presence of *p*-hydroxyphenyl moiety (δ 129.6, 127.9×2, 115.4×2, 157.2), C-2, 3, 4 carbons of flavanone skelton (δ 79.3, 43.7, 192.3, respectively) and 6,7-dihydroxy-A-ring of flavanone skeleton (δ 99.2, 110.0, 113.3, 141.2, 155.2, 157.6). From these data, the structures of **9** was elucidated to be 6,7,4′-trihydroxyflavanone. The ^{13}C-NMR assignment also supported the structure. The ^{1}H-NMR and ^{13}C-NMR spectra of **9a** gave the same signal profiles as those of **9**. The absolute configuration at C-2 was elucidated as 2*S* from the sign of CD spectrum ($[\theta]_{348}$ +4,140, $[\theta]_{313}$ − 7,100).[11]

10 gave the molecular formula, $C_{16}H_{14}O_5$, from the FAB-MS; m/z 287 [MH]$^+$. Acetylation of **10** gave diacetate (**10a**), $C_{20}H_{18}O_7$. The ^{1}H-NMR spectrum of **10** showed the almost same signal pattern with that of **9** except presence of methoxyl group [δ 3.78 (3H, s)]. From these facts, **10** was supposed to be monomethyl ether of **9**. The position of methoxyl group was determined from the NOE experiment. The irradiation at the methoxyl group induced its correlation with H-8 [δ 6.39 (1H, s)] (Fig. 1). From these data, the structure of **10** was established to be 6,4′-dihydroxy-7-methoxyflavanone. The ^{13}C-NMR spectrum also supported the

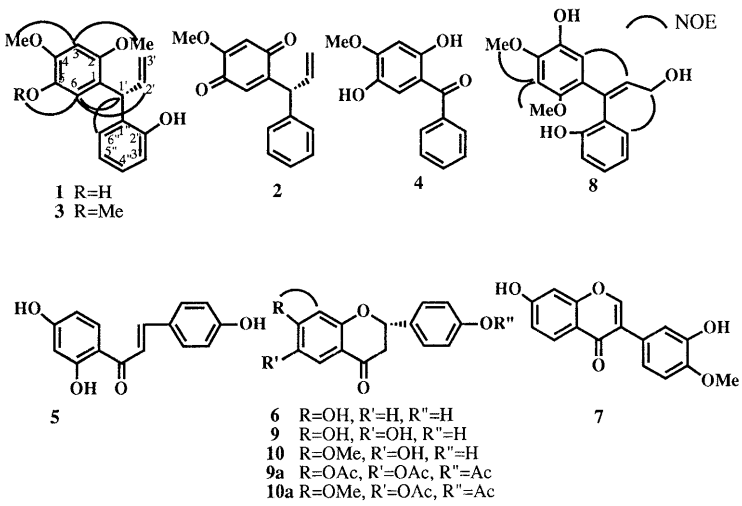

Fig. 1. Structures of the Constituents and NOEs of **3**, 8 and **10**

TABLE I. ^{13}C-NMR Data of Neoflavone Derivatives

	4 [a]	8 [b]
C-1	111.8	127.0
2	160.2	152.8
3	100.0	98.3
4	153.9	139.7
5	138.3	146.9
6	116.8	115.6
1′	20.1	135.9
2′	—	131.9
3′	—	60.1
1″	137.8	124.4
2″	128.4	150.1
3″	128.6	116.4
4″	131.5	128.4
5″	128.8	129.7
6″	128.4	129.7
OMe	56.3	56.0×2

[a] In $CDCl_3$, [b] in $CDCl_3+CD_3OD$.

TABLE II. ^{13}C-NMR Data of **6**, **9** and **10** (in $CDCl_3+CD_3OD$)

	6	9	10
C-2	79.5	79.6	79.3
3	43.7	43.6	43.7
4	192.0	192.4	192.3
5	128.8	110.2	110.0
6	110.7	140.3	141.2
7	164.9	154.1	155.2
8	102.8	103.1	99.0
4a	113.7	112.7	113.3
8a	163.9	157.8	157.7
1′	129.6	129.7	129.6
2′	127.7	127.6	127.8
3′	115.3	115.2	115.4
4′	157.2	157.1	157.2
5′	115.3	115.2	115.4
6′	127.7	127.6	127.8
OMe			55.9

TABLE III. Inhibitory Effects of Constituents of *Dalbergia cochinchinensis* on 5α-Reductase and on Formation of 5α-DHT-Receptor Complex at 100 μg/ml

Test compound	Inhibitory effect (%) on 5α-reductase	Inhibitory effect (%) on 5α-DHT-receptor complex formation
1	8.8	76.6
2	69.9	85.3
3	28.1	51.6
4	13.2	15.5
5	73.0	59.0
6	24.0	44.6
7	79.4	14.0
8	0.4	35.8
9	0	31.0
10	12.6	43.6

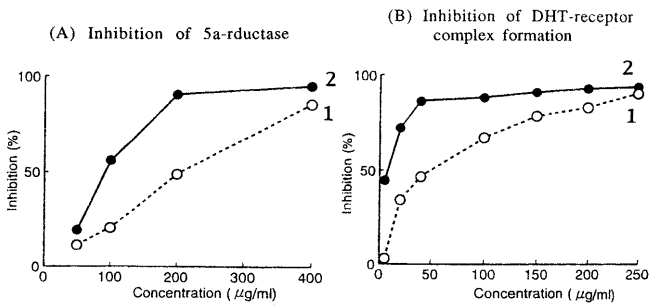

Fig. 2. Inhibitory Effects of **1** and **2** on 5α-Reductase and on Formation of DHT-Receptor Complex

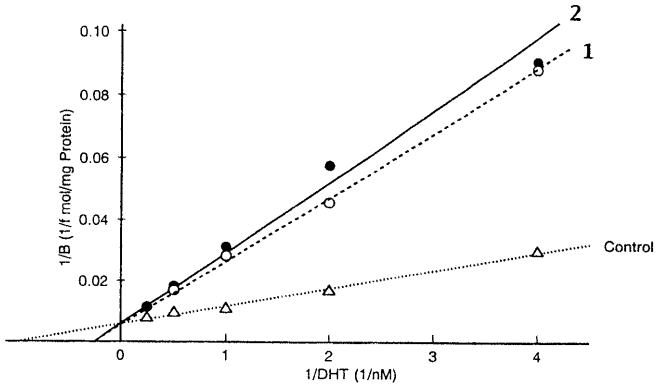

Fig. 3. Lineweaver-Burk's Double Reciprocal Plots of [^{3}H]DHT Binding in the Presence of **1** and **2**

structure (TABLE II). ^{1}H-NMR and ^{13}C-NMR spectra of **10a** gave the same signal profiles as those of **10**. The absolute configuration of **10** was established to be 2*S* from the CD spectrum ($[\theta]_{350}$ +6,360,$[\theta]_{313}$ −9,140).[11]

The isolated compounds were tested for the inhibitory activities on 5α-reductase and on the DHT binding to its receptor. The results were shown in TABLE III. Of these compounds, **2**, **5** and **7** showed potent inhibitory activities on testosterone 5α-reductase and **1** and **2** showed potent inhibitory activities on DHT binding to its receptor. Of these active compounds, the main constituents **1** and **2** were examined for the inhibitory activities at various concentrations as shown in Fig. 2. The benzoquinone type derivative, **2**, was more active than **1** in the two inhibitory tests and both compounds showed more potent inhibitory activity in DHT binding to its receptor than in 5α-reductase. The quantitative analysis of the binding of DHT to its receptor at various [^{3}H]DHT concentrations by the double reciprocal plot of Lineweaver-Burk shown in Fig. 3 indicated that both **1** and **2** inhibited the binding in a competitive manner.

Acknowledgements: We are grateful to Dr. M. Uchida of the Central Analytical Center of this university for measurement of FAB-MS. This work was supported by the Japan Health Sciences Foundation.

REFERENCES

1) N. Bruchovsky, J. D. Wilson, *J. Biol. Chem.*, **243**, 2012 (1968).
2) J. Strauss, D. Downing, F. J. Ebling, "Biochemistry and Physiology of the Skin," Vol. 1, ed. by L. A. Goldsmith, Oxford University Press, Oxford, 1983.
3) K. Bingham, D. A. Shaw, *J. Endocrinol.*, **57**, 111 (1973).
4) P. K. Siiteri, J. D. Wilson, *J. Clin. Invest.*, **49**, 1737 (1970).
5) C. B. Dempsey, D. M. X. Donnelly, R. A. Laidlaw, *Chem. Ind.*, 491 (1993).
6) D. M. X. Donnelly, T. O. Criodain, M. O'Sullivan, *Proc. R. Ir. Acad.*, **83B**, 39 (1983).
7) W. B. Eyton, W. D. Ollis, I. O. Sutherland, O. R. Gottlieb, M. T. Magalhaes, L. M. Jackman, *Tetrahedron*, **21**, 2683 (1966).
8) D. M. X. Donnelly, B. J. Nangle, J. P. Prendergst, A. M. O'Sullivan, *Phytochemistry*, **7**, 647 (1968).
9) M. T. Saliganc de Souza Guimaraes, *Phytochemistry*, **13**, 292 (1974).
10) G. D. Manners, L. Jurd, K. L. Stevens, *Phytochemistry*, **13**, 292 (1974).
11) W. Gaffield, *Tetrahedron*, **26**, 4093 (1970).