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ORIGINAL ARTICLE

Jie Liu · Ryo Ando · Kuniyoshi Shimizu · Koh Hashida
Rei Makino · Seiji Ohara · Ryuichiro Kondo

Steroid 5α-reductase inhibitory activity of condensed tannins from woody plants

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Abstract In humans, steroid 5α-reductase is involved in the development of benign prostatic hyperplasia (BPH). We tested the steroid 5α-reductase inhibitory activity of the 70% acetone extract of woody plants. The tannin polymer prepared from the 70% acetone extract showed the highest steroid 5α-reductase inhibitory activity. Furthermore, the steroid 5α-reductase inhibitory activity of tannins was not affected by the addition of superoxide dismutase or catalase. It was concluded that the steroid 5α-reductase inhibitory activity of condensed tannin was caused by binding to the steroid 5α-reductase, rather than from the peroxide/superoxide produced by tannins. The tannins, prepared from tree barks, with potential for steroid 5α-reductase inhibitory activity might be advantageous in therapy for steroid 5α-reductase diseases such as BPH or prostate cancer.

Key words 5α-Reductase · Tannins · Bark

Introduction

The microsomal enzyme steroid 5α-reductase (EC 1.3.99.5) catalyzes the NADPH-dependent reduction of the $\Delta^{4,5}$ double bond of a variety of 3-oxo-Δ^4 steroids.[1,2] It is well documented that androgen-responsive tissues such as the prostate, seminal vesicle, epididymis, and skin metabolize the conversion of testosterone to 5α-dihydrotestosterone (DHT).[3,4] This process amplifies the androgenic response, perhaps because of the higher affinity of the androgen receptor for DHT than for testosterone.[5] Both steroid 5α-reductase and DHT perform critical roles physiologically and pathologically in humans. For example, DHT is necessary for adult prostate enlargement[6] and for the development of male genitalia and normal beard growth,[7] while administration of DHT can enlarge the undetectable prostate[8] of men who were born with a genetic steroid 5α-reductase deficiency.[9] The plasma level of DHT has been reported to be elevated in patients with either benign prostatic hyperplasia (BPH) or prostatic cancer.[10] Therefore, inhibition of androgen action by steroid 5α-reductase inhibitors is a logical treatment of steroid 5α-reductase activity disorders.

The study of the inhibition of steroid 5α-reductase with organic molecules has lasted more than two decades; consequently, numerous steroidal and nonsteroidal compounds have been designed and synthesized as competitive, noncompetitive, and uncompetitive inhibitors of steroid 5α-reductase. Among them, benzoquinolinones[11,12] and 4-azasteriods[13,14] have high inhibitory potencies to either the type 1 and/or the type 2 enzyme(s) in vitro and/or in vivo. Finasteride, a synthetic steroid 5α-reductase inhibitor, is currently used to treat BPH.[15] However, these inhibitors may cause adverse effects, such as gynecomastia, impaired muscle growth, and severe myopathy.[16]

Beginning in 1960, with the increase in the demand for wood, wood-yard residues like tree bark have been generated during the handling of logs before their introduction into sawmilling or pulping facilities. For many years, burners and their smoke emissions were a familiar part of the landscape, because much of this woody waste was disposed of in burners or buried in landfills. Due to increasing concern with air quality, these burners are being phased out, while increasing disposal costs and environmental concerns are also reducing the opportunities for landfilling.[17] In particular, the reuse of the tree bark, of which 7.2 million m^3 is produced every year, is desirable.[18]

Tannins are plant polyphenolic compounds that are subdivided into two groups based on chemical structure: hydrolysable tannins and condensed tannins. The tree bark is a

J. Liu · R. Ando · K. Shimizu · R. Kondo (✉)
Department of Forest and Forest Products Science, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan
Tel. +81-92-642-2811; Fax +81-92-642-2811
e-mail: kondo@agr.kyushu-u.ac.jp

K. Hashida · R. Makino · S. Ohara
Forestry and Forest Products Research Institute, Tsukuba 305-8687, Japan

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good source of condensed tannins, and the extraction yield of tree bark is usually higher than that of wood. Both groups tend to have similar biological properties. Hydrolysable tannins are present in plants as gallotannins or ellagitannins. Gallotannins consist of a central molecule, such as glucose, surrounded by gallic acid units, for example, pentagalloylglucose. Oxidative coupling of neighboring gallic acid units or oxidation of aromatic rings is responsible for structural variation and the production of large and complex hydrolysable tannins.[19] Condensed tannins, the most common type of tannins, comprise a group of polyhydroxyflavan-3-ol oligomers and polymers linked by carbon–carbon bonds between flavanol subunits.

Tannins are primarily known for their free-radical scavenging and antioxidant activity.[20–23] However, these compounds have also been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory, antiallergic, and vasodilatory actions.[24,25] In addition, they have been reported to inhibit lipid peroxidation, platelet aggregation, capillary permeability, and fragility, and to affect enzyme systems including phospholipase A2, cyclooxygenase, and lipoxygenase.[20,24–26] These varied biological activities have resulted in the phytopharmaceutical application of tannins in the reduction of edema, increase of peripheral circulation, improvement in vision, treatment of diabetic retinopathy, prevention of cardiovascular disease, treatment of hypercholesterolemia, stabilization of connective tissue tone, reduction of adverse allergic and inflammatory responses, and enhancement of immune function and wound healing. The reactivity of condensed tannins with molecules of biological significance has important nutritional and physiological consequences. Their multiple phenolic hydroxyl groups lead to the formation of complexes with proteins, metal ions, and other macromolecules like polysaccharides. The key feature that gives tannins their characteristic properties seems to be an unusually high local concentration of *ortho*-phenolic hydroxyl groups. These are on gallic and ellagic acid in hydrolysable tannins and on B-ring phenolic hydroxyls and possibly esterified gallates in condensed tannins.[27]

Based on these reported findings, condensed tannins from tree bark may be a useful component in the treatment of a number of conditions. In this article, we describe the 5α-reductase inhibitory activity of condensed tannins from tree bark for the first time.

Materials and methods

Plant materials

Bark samples were prepared from seven tree species grown in four countries. *Acacia mangium* (Willd.) (Leguminosae) was collected in Penang, Malaysia, *Sonneratia caseolaris* in Thai Binh, Socialist Republic of Vietnam, *Acacia mearnsii* (Leguminosae) in Kumamoto, Japan, *Salix rorida* (Salicaceae) in Hokkaido, Japan, *Larix leptolepis* (Pinaceae) in Iwate, Japan, *Cryptomeria japonica* (Taxodiaceae) in Fukuoka, Japan, and *Thujopsis dolabrata* var. *homadae* (Cupressaceae) in Aomori, Japan. Bark samples were air-dried and ground in a Wiley mill.

Unless otherwise specified, chemicals were obtained from Sigma Aldrich (Tokyo, Japan). Organic solvents, superoxide dismutase (SOD), and catalase were purchased from Wako (Osaka, Japan). [4-^{14}C] Testosterone was obtained from Perkin Elmer (Kanagawa, Japan).

Fractionation of tannins

Bark meals of seven species were extracted three times with 70% acetone aqueous solution at room temperature overnight. The extract was recovered by filtration. Acetone was removed on a rotary evaporator, and each aqueous solution was freeze-dried to give a crude extract (CE). In addition, two commercial extracts of quebracho (*Schinopsis lorentzii*) (Fuji Chemical, Japan) and chestnut (*Castanea sativa* extract, Tanin Sevnica, Slovenia) were used as CE. CE was suspended in water and extracted with hexane to remove fats. The aqueous layer was extracted five times with ethyl acetate. The ethyl acetate-soluble fraction was dried on a rotary evaporator to give ethyl acetate extract (EA). Methanol (MeOH) was added to the aqueous layer to give 50% (v/v) MeOH aqueous solution. The solution was applied to Sephadex LH-20 column chromatography and was eluted with MeOH/H_2O (1/1, v/v) until the eluate was clear. The solution was then eluted with acetone/H_2O (1/1, v/v) to recover an acetone/water-soluble fraction (AW).

Characterization of flavanol monomers constituting tannins

Characterization of flavanol monomers constituting condensed tannins was conducted by measuring ^{13}C nuclear magnetic resonance (NMR) spectra of AW. These spectra were recorded on a Jeol ALPHA-500 NMR spectrometer at room temperature. Acetone-d_6-D_2O (9/1, v/v) was used as solvent.

Measurement of molecular weight distribution of tannins

Tannins (EA and AW) were acetylated with pyridine–acetic anhydride (1/1, v/v) at room temperature for 2 days, and the acetylated samples were subjected to gel permeation chromatography (GPC). GPC was performed with a Shim-pack GPC-803 column (300 × 8 mm i.d., Shimadzu, Japan) using a LC-VP system (Shimadzu, Japan). Analysis conditions were as follows: mobile phase, tetrahydrofuran; flow rate, 1 ml/min; column temperature, 40°C; detection, ultraviolet absorbance at 280 nm. The average molecular weight was determined from a calibration curve prepared with standard polystyrenes.

Preparation of rat microsome samples

Rat liver microsome samples from female Sprague-Dawley (SD) rats (7 weeks old) were prepared by the modified

method previously reported by Shimizu et al.[28] with some modifications. Two mature female SD rats were killed. The liver was removed, and minced tissue was then homogenized in a four-tissue volume medium A (0.32 M sucrose, 1 mM dithiothreitol, and 20 mM sodium phosphate, pH 6.5). The homogenate was then centrifuged at 10 000 *g* for 10 min. The resulting supernatant from the centrifugation was further centrifuged twice at 105 000 *g* for 1 h. The washed microsome samples were suspended in one-pellet volume medium A, and the dispersion of microsome samples was achieved using a syringe with 18 G, 23 G, and 26 G needles in succession. The microsome suspension was divided, diluted with medium A, and stored at –80°C until just before use.

Measurement of 5α-reductase inhibitory activity

The complete reaction mixture included 1 mM dithiothreitol, 20 mM phosphate buffer (pH 6.5), 1.9 nCi [4-^{14}C] testosterone, 150 μM testosterone, 167 μM NADPH, and the enzyme preparation (1.54 mg of protein) in a final volume of 0.3 ml. The concentration of testosterone contributed by [4-^{14}C] testosterone was negligible. Each of the extracts were added to the solution at each concentration [dimethyl sulfoxide (DMSO) was used as the solvent, and final concentration of the DMSO was 4.5%].The incubation was carried out for 10 min at 37°C and was started by the addition of 10-μl microsome samples to the preheated reaction solution in a tube. After 10 min, the incubation was terminated by adding 10 μl of 3 M NaOH. To extract the metabolites, 1 ml of diethyl ether was added, and the tubes were capped and shaken. The organic phase was applied to a silica plate (Kieselgel 60 F_{254}). The plate was developed with ethyl acetate–hexane (7:3) at room temperature. The radioactivity profile was determined with an imaging analyzer (FLA-5000 RF, Fuji Film, Tokyo, Japan). The 5α-reductase activity was calculated from the percentage of the extent of the conversion of [4-^{14}C] testosterone to [4-^{14}C] dihydrotestosterone.

Preincubation assay of steroid 5α-reductase inhibitory activity

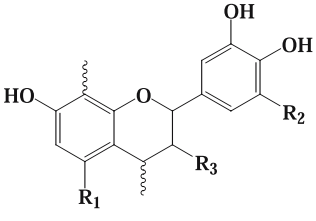
A reaction mixture included 1 mM dithiothreitol, 20 mM phosphate buffer (pH 6.5), 1.9 nCi [4-^{14}C] testosterone, 150 μM testosterone, and 167 μM NADPH in a final volume of 0.23 ml. The concentration of testosterone contributed by [4-^{14}C] testosterone was negligible. The preincubation of tannins (DMSO was used as the solvent, and final concentration of the DMSO was 4.5%) and the microsome samples (1.54 mg of protein) was carried out for 10 min at 37°C, and then the preincubation solution was added to the preheated reaction mixture in a tube. After 10 min, the incubation was terminated by adding 10 μl of 3 M NaOH. To extract the metabolites, 1 ml of diethyl ether was added, and the tubes were capped and shaken. The organic phase was applied to a silica plate (Kieselgel 60 F_{254}), which was developed with ethyl acetate–hexane (7:3) at room temperature. The radioactivity profile was determined with an imaging analyzer (FLA-5000 RF, Fuji Film). The 5α-reductase activity was calculated from the percentage of the conversion of [4-^{14}C] testosterone to [4-^{14}C] dihydrotestosterone.

Determination of hydrogen peroxide generated by condensed tannins

Each tannin fraction was dissolved in DMSO. A reaction mixture (Eagle's minimal essential media; EMEM) that included 100 μg/ml condensed tannins was incubated for 1 h (final concentration of DMSO was 0.1%). The reaction mixture (300 μl) was added to 1 ml ferrous oxidation-xylenol orange reagent [FOX; $FeSO_4 \cdot 7H_2O$ (250 μM), 2,6-di-*tert*-butyl-4-methylphenol (4 mM), H_2SO_4 (25 mM), xylenol orange (76 mg/ml)], and then incubated at room temperature for 30 min. After incubation, the mixture was centrifuged at 20 000 *g* for 10 min and the absorbance of the resulting supernatant at 560 nm was measured.[29]

Results and discussion

The structures of tannins in EA and AW are listed in Table 1 and shown in Fig. 1, and the yields of CE, EA, and AW are shown in Table 2. Structural characterization of AW was based on ^{13}C NMR spectroscopic analysis and the results are shown in Fig. 2. The chemical shifts of these spectra were assigned by comparison with literature data,[30–33] and the tannins were identified as several kinds of proanthocyanidins. Assignments of the characteristic signals are as follows. The spectra of tannins from *Acacia mangium* and *Acacia mearnsii* showed a signal at 103 ppm, which was assigned to the tertiary C-8 of the resorcinol-type A-ring, and a signal at 108 ppm, which was assigned to C-2′ and C-6′ of the pyrogallol-type B-ring.[30,31] Therefore, it was concluded that these tannins consist mainly of prorobinetinidin.[30] The spectrum of tannins from *Salix rorida* showed a signal at 98 ppm, which was assigned to the tertiary C-6



PR : Prorobinetinidin R1:H, R2:OH, R3:OH
PC : Procyanidin R1:OH, R2:H, R3:OH
PD : Prodelphinidin R1:OH, R2:OH, R3:OH
PD Gallate R1:OH, R2:OH, R3:O-Galloyl
PF : Profisetinidin R1:H, R2:H, R3:OH

Fig. 1. Chemical structures of condensed tannins

Table 1. Structural properties of tannin fractions from tree bark

Tree species	EA			AW			Main structures
	M_n	M_w	M_w/M_n	M_n	M_w	M_w/M_n	
Acacia mangium	1300	1900	1.5	2500	3100	1.2	PR
Acacia mearnsii	1300	1800	1.4	2100	3200	1.5	PR
Salix rorida	1100	2400	2.2	3000	5500	1.8	PC/PD*
Cryptomeria japonica	660	1900	2.9	2800	11300	4	PC
Larix leptolepis	680	1400	2.1	3900	14800	3.8	PC
Thujopsis dolabrata	970	1500	1.5	2600	4900	1.9	PC
Sonneratia caseolaris	350	700	2	3300	6600	2	PD, PD gallate
Quebracho	950	1400	1.5	2100	2800	1.3	PF
Chestnut	420	840	2	2300	3400	1.5	Ellagitannin

EA, Ethyl acetate extract; AW, acetone-water (1:1, v/v) eluates; M_n, number-average molecular weight; M_w, weight-average moleuclar weight; PR, prorobinetinidin; PC, prodelphinidin; PD, gallate; PF, profisetinidin

*Proanthocyanidin which contains both procyanidin- and prodelphindin-type units

Table 2. Extraction yield of each fraction

Tree species	CE[a] (%)	EA[b] (%)	AW[b] (%)
A. mangium	37.9	42.2	20.7
A. mearnsii	52.7	50.5	23.7
S. rorida	34.9	8.8	46.7
C. japonica	7.5	8.8	1.0
L. leptolepis	10.4	31.3	32.3
T. dolabrata	17.8	14.8	11.2
S. caseolaris	28.2	4.7	32.5
Quebracho		15.3	12.3
Chestnut		8.2	15.5

CE, Crude extract

[a] Percentage based on tree bark

[b] Percentage based crude extract

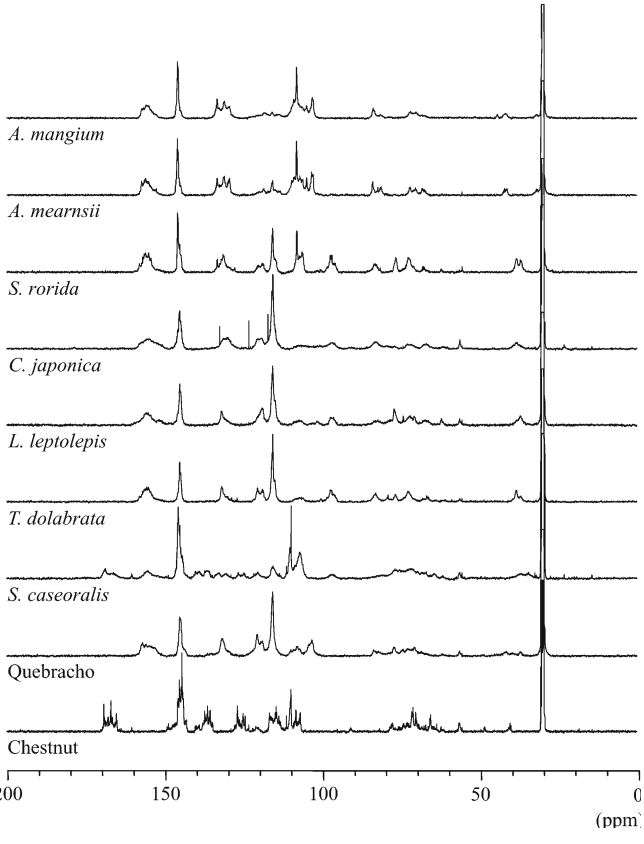


Fig. 2. ^{13}C Nuclear magnetic resonance spectra of acetone-water (1:1, v/v) eluates (AW) fractions from tree bark

or C-8 of the phloroglucinol-type A-ring, and a signal at 115 ppm, which was assigned to C-2′ and C-5′ of the catechol-type B-ring, together with the carbon of the pyrogallol-type B-ring (108 ppm). This result indicates tannins from *S. rorida* are proanthocyanidins that contain both procyanidin- and prodelphinidin-type units.[31] Tannins from *Larix leptolepis*, *Cryptomeria japonica*, and *Thujopsis dolabrata* were concluded to contain procyanidin,[32] because of the presence of signals assigned to the carbons of the phloroglucinol-type A-ring (98 ppm) and the catechol-type B-ring (115 ppm). The spectrum of tannins from quebracho showed carbon signals of the resorcinol-type A-ring (103 ppm) and the catechol-type B-ring (115 ppm); thus, these tannins consist of profisetinidin.[33] The ^{13}C NMR spectrum of *Sonneratia caseolaris* showed carbonyl carbon (167 ppm) and aromatic carbon (110 ppm) signals, which were assigned to galloyl groups attached to flavanol monomers, in addition to those assigned to the prodelphinidin extender unit (97 and 108 ppm). Therefore, tannins from *S. caseolaris* were assumed to consist of prodelphinidin 3-*O*-gallate and prodelphinidin extender units, similar to persimmon tannins.[34] The main components of tannins from chestnut were revealed to be ellagitannins (hydrolysable tannin) as previously described.[35]

Steroid 5α-reductase inhibitory activity was assayed on CE, EA, and AW samples. In this screening assay for the fractions of each tree bark, all the CE, EA, and AW showed steroid 5α-reductase inhibitory activity, and AW of each tree bark showed the highest inhibitory activity (Table 3). The IC_{50} of AW ranged from 39 to 75 μg/ml, and that of EA ranged from 90 to 130 μg/ml. These results suggest that the extracts of tree barks can inhibit the 5α-reductase. It should be noted that α-linolenic acid, the natural compound with steroid 5α-reductase inhibitory activity that was used as a positive control, showed an IC_{50} of 116 μM (32 μg/ml) in our assay system.[36]

As shown in Table 1, EA and AW of *A. mearnsii* and *A. mangium* contain the prorobinetinidin structure; *S. rorida*,

Table 3. Effects of CE, EA, AW, and some polyphenols on steroid 5α-reductase activity

Sample	IC_{50} (μg/ml)			
	CE	EA	AW	Polyphenol
A. mangium	80	130	52	
A. mearnsii	81	90	69	
S. rorida	87	105	53	
C. japonica	92	95	39	
L. leptolepis	82	83	54	
T. dolabrata	96	120	42	
S. caseolaris	56	93	47	
Quebracho	153	94	43	
Chestnut	63	93	75	
α-Linolenic acid (positive control)				32 (116μM)
(+)-Catechin				-[a]
(−)-Epicatechin				-[a]
(−)-Epigallocatechin				-[a]
(−)-Epigallocatechin gallate				-[a]
Gallic acid				-[a]

IC_{50}, Concentration causing 50% inhibition
[a] No inhibition at 75μg/ml

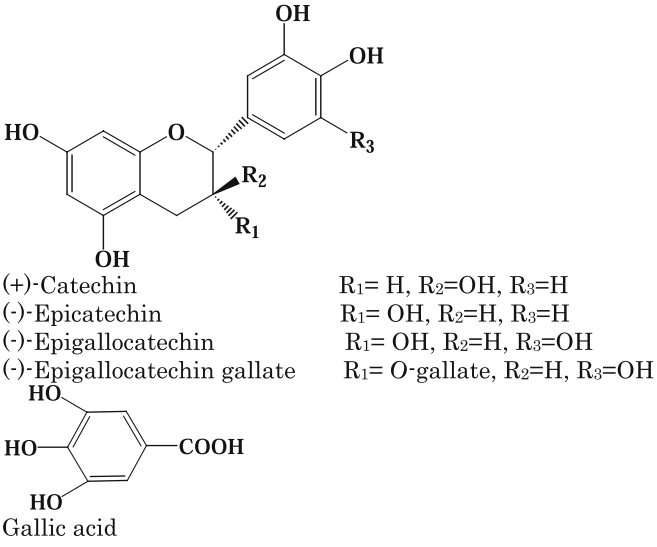


Fig. 3. Structures of polyphenols tested for inhibitory activity against steroid 5α-reductase

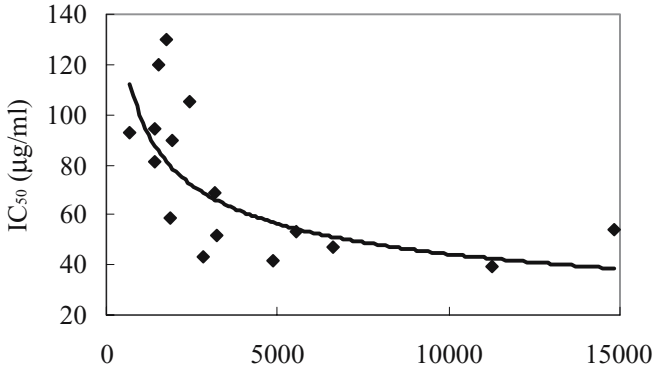


Fig. 4. The relationship of weight average molecular weight of condensed tannins and the IC_{50} (50% inhibitory concentration) of steroid 5α-reductase inhibitory activity

L. leptolepis, C. japonica, and *T. dolabrata* contain the procyanidin structure; and *S. caseolaris* contains the prodelphinidin gallate structure. Each fraction also has a different molecular weight and polymerization degree. To determine what part of the substructure of tannins was important for the inhibition of 5α-reductase, we tested some natural flavonoids for their ability to inhibit steroid 5α-reductase. In contrast to the result for tannins, four flavonoids, (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, and (−)-epigallocatechin gallate (Fig. 3), showed no inhibitory activity against steroid 5α-reductase at 75μg/ml (Table 3).

No effect of the substructure on the steroid 5α-reductase inhibitory activity was observed. For example, the AW of *C. japonica* had the lowest IC_{50} among all the AW fractions, but the EA and CE of *C. japonica* showed higher IC_{50} values than those of *A. mearnsii* or *L. leptolepis*. From Table 3, the IC_{50} sequence of the steroid 5α-reductase inhibitory activity is EA > CE > AW, while the molecular weight and polymerization degree of EA is lower than those of AW. As shown in Fig. 4, the molecular weight of each fraction is related to the steroid 5α-reductase inhibitory activity. With increasing molecular weight, the IC_{50} of steroid 5α-reductase inhibitory activity rapidly decreased, although no remarkable decrement was observed for molecular weight over 5000. From the results of Table 3 and Fig. 4, it seems that the inhibitory activity of the condensed tannins on steroid 5α-reductase is related to the molecular weight and the polymerization degree. It is widely accepted that condensed tannins react with various enzymes rendering them inactive. For example, tannins cause the precipitation of β-glucosidase (EC 3.2.1.21) from solution by forming a tannin–enzyme complex and the enzyme activity is decreased.[37–39] Haslam[37] suggested that the inhibition of β-glucosidase was due to precipitation, not to inhibition of the enzyme by residual soluble phenols. Bate-Smith[40] reported that the astringency of tannins, that is their efficiency as precipitants of proteins, increased progressively from low molecular weight to high molecular weight. Our present

results support these reports. Figure 4 and Table 3 show that the activity of tannins to inhibit steroid 5α-reductase was dependent on the degree of polymerization. Thus, although all enzymes were not subjected to the determination of protein-precipitating capacity, it seems reasonable to assume that the loss of enzyme activity observed in the present experiments is principally due to the formation of an insoluble enzyme–tannin complex.

Certain flavonoids show inhibition against a variety of enzymes.[40,41] Some properties of these compounds may be responsible for their biological activity, such as their ability to form complexes with certain metal ions or proteins, and their antioxidant and pro-oxidant activities. It does not appear likely that metal ion complexation would be responsible for the inhibition of steroid 5α-reductase because of the lack of metal ions in 5α-reductase.

Certain flavonoids produce hydrogen peroxide in aqueous solutions at physiological pH, possibly through a superoxide intermediate.[42,43] From ^{13}C NMR data, we saw that tannins from *C. japonica* consisted of catechol-type B-rings. In our hydrogen peroxide generation assay, tannins from *C. japonica* generated 20 μM hydrogen peroxide in 1 h. To determine whether these reactive oxygen species may have some role in the inhibition of steroid 5α-reductase by condensed tannins, we added SOD or catalase to assay mixtures containing condensed tannin samples. All of these proteins had the same protein content as the active steroid 5α-reductase. We knew that if the inhibition of the tannins on 5α-reductase was not a specific inhibition, the percentage of inhibitory activity would be changed by this addition. However, the addition of these enzymes did not affect the inhibition of 5α-reductase (data not shown). Therefore, peroxide and superoxide do not appear to be responsible for the inhibition of 5α-reductase by condensed tannins.

To investigate whether the inhibition activity of tannins on steroid 5α-reductase is derived only from the precipitation activity of the protein, we designed the following experiments. In these experiments, we used the tannins from *C. japonica* as the sample. In the assay for the measurement of 5α-reductase inhibitory activity, the tannins were added to the reaction solution including testosterone, NADPH, and buffer, and then incubated with the steroid 5α-reductase. In the preincubation assay, we incubated each tannin sample of *C. japonica* with the steroid 5α-reductase (preincubation) first, and then this preincubated solution was added to the reaction solution (testosterone and NADPH in buffer). After the tannins bound to the steroid 5α-reductase, the metabolism of testosterone to DHT was started. After preincubation with steroid 5α-reductase, the percentage of steroid 5α-reductase inhibitory activity increased from 65% to 84% for EA, 54% to 77% for AW, and 38% to 88% for CE (Fig. 5). Conversely, adding other protein (SOD or catalase) caused no change in the steroid 5α-reductase inhibitory activity (data not shown).

These data showed that the tannins from *C. japonica* can bind to steroid 5α-reductase, and this binding inhibits the conversion of testosterone to DHT. It is reported that tea catechins can form precipitates with soybean lipoxygenase and yeast alcohol dehydrogenase. The basis for this precipitation activity has not been defined thoroughly, but it may be due to the ability of certain polyphenols to form both numerous hydrogen bonds with a protein, as well as unselective association of the aromatic nuclei of a polyphenol with certain amino acids, especially prolines.[40] 5α-Reductase contains 14 (5.4%) proline residues; thus, this enzyme is not a proline-rich protein. Also, only 5 of these proline residues are in the carboxyl-terminal half of the protein containing the putative NADPH-binding site. From the results shown above, we see that the steroid 5α-reductase inhibitory activity of condensed tannin comes from the binding to the steroid 5α-reductase, but not from the peroxide/superoxide or the precipitation of the steroid 5α-reductase protein. Also, the inhibitory activity of condensed tannin comes in part from its selective binding ability to the steroid 5α-reductase protein because the addition of other enzymes (SOD or catalase) to the assay system did not affect its inhibition of 5α-reductase (data not shown). In other words, the condensed tannin binds to the steroid 5α-reductase protein more selectively than SOD or catalase.

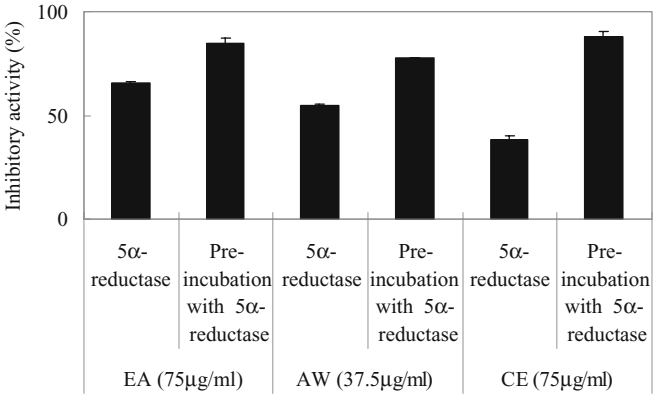


Fig. 5. The effect of preincubation of *Cryptomeria japonica* extracts with steroid 5α-reductase on steroid 5α-reductase inhibitory activity. *EA*, Ethyl acetate extract; *AW*, acetone-water (1:1, v/v) eluates; *CE*, crude extract

The proposed mechanism of the steroid 5α-reductase (E) reaction includes the following steps:

$$\mathrm{NADPH} + \mathrm{E} \leftrightarrow [\mathrm{NADPH\text{-}E}] \overset{+\mathrm{Testosterone(T)}}{\leftarrow (\mathrm{a}) \rightarrow} [\mathrm{NADPH\text{-}E\text{-}T}]$$
$$\overset{+\mathrm{H^+}}{\rightarrow} [\mathrm{NADP^+\text{-}E\text{-}DHT}]\text{-}(\mathrm{b}) \rightarrow \mathrm{DHT} + [\mathrm{NADP^+\text{-}E}]$$
$$\rightarrow \mathrm{NADP^+E}$$

Steroidal inhibitors that can inhibit either step a or step b have been found.[44–48] However, our present results do not exclude the possibility that condensed tannins inhibit the formation of [NADPH-E-T] (step a) or the formation of the ternary complex (step b). As shown in Fig. 5, it is clear that tannins can bind to steroid 5α-reductase itself to inhibit the conversion of testosterone to DHT, but we still cannot deny the possibility of inhibition during step a or b.

In this study, we found a new facet of the biological activities of the condensed tannins, steroid 5α-reductase inhibitory activity. Because excessive steroid 5α-reductase activity has been proposed as a possible contributing factor in prostate cancer development or progression,[49] the devel-

opment and progression of prostate cancer may also be affected by steroid 5α-reductase inhibitors. By modulating steroid 5α-reductase activity, the condensed tannins may have the ability to act as prostate cancer chemopreventative agents. If some of these compounds are active in vivo, they may be important candidates for prostate cancer chemopreventative agents. Also, the extracts of tree barks might be advantageous for the therapy against steroid 5α-reductase diseases. At this time, the clinical implications of this activity are unknown, so further research is needed to explore the general uses of these extracts of tree barks.

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