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A geranylated chalcone with 5α-reductase inhibitory properties from *Artocarpus incisus*

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Abstract

A geranylated chalcone was isolated from leaves of *Artocarpus incisus* and it showed potent 5α-reductase inhibitory activity.

Keywords: *Artocarpus incisus*; Moraceae; 5α-Reductase inhibitor; Geranylated chalcone; 3′-Geranyl-2′,3,4,4′-tetrahydroxychalcone

1. Introduction

Some isoprenylated compounds from *Artocarpus incisus* plants show interesting biological activities. For example, isoprenoid-substituted flavonoids and stilbenes isolated from *A. incisus* species possess inhibitory effects on melanin biosynthesis (Shimizu et al., 1998). They also inhibit 5α-reductase activity (Shimizu et al., 2000), in the amino acid transport system in Lepidoptera (Parenti et al., 1998), arachidonate 5-lipoxygenase and mouse TNF-α release (Nomura et al., 1998). Their cytotoxicity against cancer cells, antiplatelet aggregation activity in rabbit platelet suspensions, and antibacterial activities against cariogenic bacteria have also been reported (Nomura et al., 1998).

Δ^4-3-Oxo-steroid 5α-reductase (EC 1.3.99.5; 5α-reductase) inhibitors would be useful in the selective treatment of androgen-dependent abnormalities, such as benign prostate hyperplasia, prostate cancer, hirsutism, male pattern alopecia and acne. In the course of research on the isolation of biologically active substances from tropical plants, we discovered that acetone extracts of the leaves of *A. incisus* showed potent 5α-reductase inhibitory activity. The present communication describes the isolation and structural elucidation and 5α-reductase activity of a new geranylated chalcone, 3′-geranyl-2′,3,4,4′-tetrahydroxychalcone, from leaves of *A. incisus*.

2. Results and discussion

The acetone extract of leaves of *A. incisus* was partitioned by *n*-hexane, diethyl ether and ethyl acetate. The 5α-reductase inhibitory activity of the *n*-hexane-soluble, diethyl ether-soluble, ethyl acetate-soluble and aqueous portions were 20, 67, 33 and 0%, respectively at a concentration of 100 μg/ml. Since the diethyl ether-soluble portion showed the strongest 5α-reductase inhibitory activity, it was submitted to further separation by silica gel CC and then preparative HPLC, resulting in an active constituent, compound **1**.

Compound **1** was deduced as having an elemental formula of $C_{25}H_{28}O_5$ by HR–FAB mass spectrometry (observed $[M + H]^+$ m/z 409.2014). The ^{1}H NMR spectrum showed characteristic signals for protons at

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the C-α and C-β positions of a chalcone skeleton [δ 7.67, 7.75 (each 1H, *d*, $J = 15.1$ Hz)], and revealed the presence of ABC-type aromatic protons [δ 6.88 (1H, *d*, $J = 8.3$ Hz), 7.20 (1H, *dd*, $J = 2.0$, 8.3 Hz), 7.32 (1H, *d*, $J = 2.0$ Hz)], AB-type aromatic protons [δ 6.51 (1H, *d*, $J = 8.8$ Hz), 7.96 (1H, *d*, $J = 8.8$ Hz)], a geranyl group [δ 1.52 (3H, *brs*), 1.58, 1.77 (each 3H, *d*, $J =$ 0.98 Hz), 1.94, 2.02 (each 2H, *m*), 3.36 (2H, *d*, $J = 7.3$ Hz), 5.08, 5.27 (each 1H, m)], and a hydrogen bonded hydroxyl group [δ 13.9 (1H, *s*)]. In the HMBC spectrum, the H-1″ proton provided a good starting point for the assignment of the proton and carbon resonances of the geranyl function. Proton H-1″ exhibited correlations with the C-2′, C-3′, C-4′, C-2″ and C-3″ carbons. This permitted assignment of the C-3′ position of the geranyl group. Accordingly, **1** was identified as 3′-geranyl-2′,3,4,4′-tetrahydroxychalcone. The structure and all assignments were confirmed by ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY and HMBC spectroscopic methods (Table 1).

Compound **1** showed more potent inhibitory activity ($IC_{50} = 104$ μM) than α-linolenic acid ($IC_{50} = 116$ μM), known as a naturally occurring potent inhibitor (Liang and Liao, 1992). Our previous results demonstrated that a geranylated stilbene (2,3′,4,5′-tetrahydroxy-4′-geranylstilbene; chlorophorin) isolated from the heartwood of *A. incisus* also showed potent 5α-reductase inhibitory activity ($IC_{50} = 37$ μM), although a non-geranylated stilbene (2,3′,4,5′-tetrahydroxystilbene; oxyresveratrol) did not (Shimizu et al., 2000). Compared to the geranylated chalcone (**1**), the absence of the geranyl substituent (2′,3,4,4′-tetrahydroxychalcone; butein) decreased the 5α-reductase inhibitory effect ($IC_{50} = 217$ μM). These observations led us to the assumption that, in the case of **1**, the geranyl moiety enhances 5α-reductase inhibitory activity. Also, these results may support our previous assumption (Shimizu et al., 2000) that isoprenyl-derived substituents (prenyl and geranyl) may be important structural moieties as far as the 5α-reductase inhibitory activity of flavonoids and stilbenes are concerned.

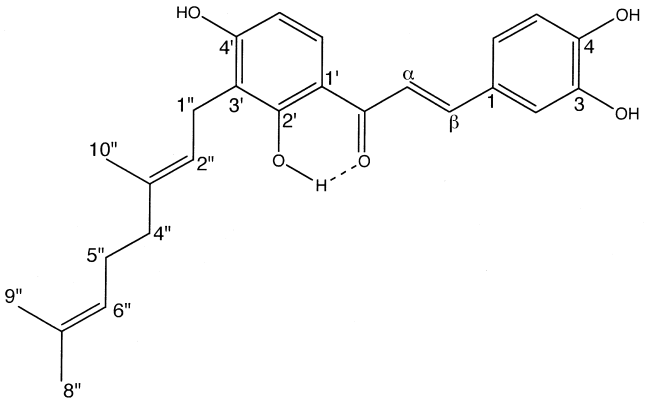

Table 1
^{1}H and ^{13}C NMR spectral data of **1** (CD_3COCD_3, 400 MHz)

Position	δ, ppm (*J*, Hz) for ^{1}H	δ (ppm) for ^{13}C
C=O		192.6
α	7.67 *d* (15.1)	118.3
β	7.75 *d* (15.1)	145.0
1		128.0
2	7.32 *d* (2.0)	115.7
3		146.1
4		148.9
5	6.88 *d* (8.3)	116.2
6	7.20 *dd* (2.0, 8.3)	123.1
1′		114.2
2′		164.9
3′		115.9
4′		162.4
5′	6.51 *d* (8.8)	107.8
6′	7.96 *d* (8.8)	129.9
1″	3.36 *d* (7.3)	22.2
2″	5.27 *m*	123.0
3″		134.9
4″	1.94 *m*	40.5
5″	2.02 *m*	27.4
6″	5.07 *m*	124.9
7″		131.3
8″	1.58 *d* (0.98)	25.8
9″	1.52 *brs*	17.7
10″	1.77 *d* (0.98)	16.3
2′-OH	13.9 *s*	

3. Experimental

NMR: 400 MHz on Jeol JNM-AL400 FT NMR spectrometer using CD_3COCD_3 as the solvent and TMS as standard reference. 2′,3,4,4′-Tetrahydroxychalcone (butein), isolated from the heartwood of *Rhus javanica*, was provided by the Laboratory of Wood Chemistry, Department of Forest Products, Faculty of Agriculture, Kyushu University in Japan; its purity and identification had been confirmed by comparison with Imamura et al. (1967).

3.1. Plant material

Leaves of *A. incisus* were collected in March 1999, Okinawa, Japan by Dr. K. Kinjyo of Ryukyu University, who confirmed its identification. The voucher specimen is preserved at the herbarium of Department of Forest Products, Kyushu University in Japan.

3.2. Extraction and isolation

Chopped leaf tissue (1.2 kg) was extracted repeatedly with acetone at room temperature. The extracts were concentrated in vacuo to give a residue (47 g). An almost of the acetone extract (10 g) was suspended

in water and partitioned with *n*-hexane, diethyl ether and ethyl acetate, successively to give *n*-hexane-soluble (1.5 g, 15% of the acetone extract), diethyl ether-soluble (7.2 g, 72%) and aqueous (1.3 g, 13%) portions. The diethyl ether-soluble portion (5 g) was fractionated by column chromatography on silica gel (500 g) eluting with an EtOAc-*n*-hexane gradient. The fraction (0.5 g) obtained with 70–90% EtOAc in *n*-hexane was subsequently subjected to preparative HPLC (Inertsil PREP-ODS: 20 mm i.d. × 250 mm) eluting with H_2O/CH_3CN (30/70), 12 ml/min, to give **1** (239 mg).

3.3. 3′-Geranyl-2′,3,4,4′-tetrahydroxychalcone (*1*)

Yellow powder; HR-FABMS: $[M + H]^+$ m/z 409.2014 ($C_{25}H_{29}O_5$ requires m/z 409.2015); ^1H and ^{13}C NMR spectral data : see Table 1.

3.4. 5α-Reductase assay

5α-Reductase inhibitory activity was assayed by the method previously reported (Shimizu et al., 2000). The reaction mixture, in a final volume of 3.0 ml, contained microsomes from female rat liver, 150 μM testosterone in 100 μl of ethanol, 167 μM NADPH, 20 mM sodium phosphate buffer (pH 6.5), with or without a sample in 100 μl of DMSO. The reaction was started by the addition of microsomes to the preheated reaction solution in a tube. After 10 min the incubation was terminated by adding 100 μl of 3 M NaOH, and then 100 μl of 1.0 mM cholesterol acetate in *n*-hexane was added as internal standard for GC–MS. To extract metabolites, 40 ml of diethyl ether was added, and the tubes were capped and shaken. The water phase was frozen at −20°C the organic phase was decanted and evaporated under reduced pressure. The residue was dissolved in 100 μl EtOAc for GC–MS. α-Linolenic acid was used as a positive standard.

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References

Parenti, P., Pizzigoni, A., Hanozet, G., Hakim, E.H., Makmur, L., Achmad, S.A., Giordana, B., 1998. A new prenylated flavone from *Artocarpus champeden* inhibits the K^+-dependent amino acid transport in *Bombyx mori* Midgut. Biochem. Biophys. Res. Commun. 244, 445–448.

Imamura, H., Kurosu, H., Takahashi, T., 1967. The chemistry of wood extractives. XVIII. The heartwood constituents of *Rhus javanica* L. Mokuzai Gakkaishi 13, 295–299.

Liang, T., Liao, S., 1992. Inhibition of steroid 5α-reductase by specific aliphatic unsaturated fatty acids. Biochem. J. 285, 557–562.

Nomura, T., Hano, Y., Aida, M., 1998. Isoprenoid-substituted flavonoids from Artocarpus plants (Moraceae). Heterocycles 47, 1179–1205.

Shimizu, K., Fukuda, M., Kondo, R., Sakai, K., 2000. The 5α-reductase inhibitory components from heartwood of *Artocarpus incisus*: structure–activity investigations. Planta Med. 66, 16–19.

Shimizu, K., Kondo, R., Sakai, K., Lee, S.H., Sato, H., 1998. The inhibitory components from *Artocarpus* incisus on melanin biosynthesis. Planta Med. 64, 408–412.