

Studies on the Constituents of the Stem Barks of *Acanthopanax gracilistylus* W. W. Smith

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Abstract – Ten compounds were isolated from the stem barks of *Acanthopanax gracilistylus* WW Smith (AGS) by steam distillation, they were p-menta-1,5,8-triene, n-butyl isobutylphthalate, p-mentha-1,5-diene-8-ol, 8-hydroxy-p-cymene, myrtenol, trans-(+)-carveol, 1,3-di-tert-butylbenzene, 4-methyl-2,6-di-butylphenol, valencene and verbenone, respectively, characterized by GS-Mass spectra. And we have also extracted and isolated from the MeOH extracts of the stem barks of AGS, two compounds were obtained. On the basis of chemical and spectral evidence, they were syringin(1), β -sitosterol(2).

Keywords – *Acanthopanax gracilistylus* WW Smith, p-menta-1, 5, 8-triene, n-butyl isobutylphthalate, p-mentha-1,5-diene-8-ol, 8-hydroxy-p-cymene, myrtenol, trans-(+)-carveol, 1,3-di-tert-butylbenzene, 4-methyl-2,6-di-butylphenol, valencene and verbenone, syringin(1), β -sitosterol(2).

Introduction

Acanthopanax species belong to Araliaceae, and its root and stem barks *Acanthopanax* Cortex, are used as tonic and prophylactic in oriental herbal medication from olden times (Yook, C. S., *et al.* 1994; Yook, C. S., *et al.* 2001; Yook, C. S., 1999). Up to now, about 30 kinds of *Acanthopanax* species are found in East Asia and South Asia on the world (Ohwi, J. 1972). There are 26 species of *Acanthopanax* widely distributed in China, and the dried roots and barks of *Acanthopanax gracilistylus* W.W. Smith are listed officially in the Chinese pharmacopoeia for the treatment of paralysis, arthritis, rheumatism, lameness, and liver disease (*Chinese Pharmacopoeia* (Part one, 2000)). There are many reports on the stem and root barks of *Acanthopanax gracilistylus* and lignans and diterpene derivatives have been isolated and identified (Song, X. H., *et al.* 1983; Xiang, R. D., *et al.* 1983; Tang, Q. Y., 1995). In continuation of our systematic chemical studies of this plant, we here report essential oils from the stem barks of AGS and other components from its MeOH extracts, they are syringin that was shown anti-stress, revival of fatigue, protection from decrease of sexual desire activities (I.I. Brekhan *et al.*, 1969), and β -sitosterol that was shown antihyperlipoproteinaemic, antiandrogenic, benign prostate hyperplasia activities (Eva S. Istvan *et al.*, 2000). These

compounds were similar to those from the roots of this plant (LIU, X. Q., 2001). Thus, it maybe one of evidences on the use of the stem barks instead of its root.

Experimental

Plant Materials – The stem barks of AGS were collected in March 2001, in ChangSha, HuNan province of China and identified by Prof. ChangSoo Yook, KyungHee University.

General experimental procedures – Melting points (uncorrected) were measured using a Boetius micromelting point apparatus. IR spectra were obtained with a Hitachi 270-30 type spectrophotometer. NMR spectra were measured in MeOD on a JEOL- α -500 spectrometer and chemical shifts were relative to tetramethylsilane (TMS). Column chromatography (CC) was carried out on Silica-gel 230~400 mesh(Merck). TLC was performed on precoated silica gel 60GF254 (Merck) and RP-18F_{254S} (Merck) plates. Steam distillation apparatus is in accord with K.P. standard. GS-Mass spectra were operated on the GC5890/MSD HP5973, DB-5MS (30 m \times 0.254 mm \times 0.25 μ m) column, ccolumn temp. 70°C (1 min)~250°C (5 min), speed of raised temp.5°C/min; Inlet temp. 270°C; Resolution: 1000; Inj. Volume: 1 ml; Inj. temp. 200°C.

Steam distillation – The stem barks (50 g) of AGS was extracted 4 hrs by water steam distillation to obtain oil-like extracts which was partitioned by ether successively. Removed ether, yellowish oil was obtained. Then they were analyzed

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Table 1. TIC data of essential oils from the stem barks of *Acanthopanax gracilistylus*

No.	Rr (min)	Qual.	M ⁺	Formula	Base peak	Other main fragments	Assignment
1	7.060	90	134	C ₁₀ H ₁₄	91	134 119 109 81	<i>p</i> -mentha-1,5,8-triene
2	7.436	92	152	C ₁₀ H ₁₆ O	59	134 119 94 79 43	<i>p</i> -metha-1,5-dien-8-ol
3	7.638	93	150	C ₁₀ H ₁₄ O	43	150 135 132 117 91	8-hydroxy- <i>p</i> -cymene
4	7.812	93	152	C ₁₀ H ₁₆ O	79	152 135 119 108 91	myrtenol
5	7.968	97	150	C ₁₀ H ₁₄ O	107	150 135 91 80 41	verbenone
6	8.106	98	152	C ₁₀ H ₁₆ O	109	152 134 119 91 84	trans-(+)-carveol
7	8.510	90	190	C ₁₄ H ₂₂	175	190 147 115 91 57	1,3-tert-butylbenzene
8	11.905	97	220	C ₁₀ H ₁₆ O	205	220 191 145 57	4-methyl-2,6-di-tert-butylphenol
9	16.925	96	278	C ₁₆ H ₂₂ O ₄	149	278 223 205 104	<i>n</i> -butyl isobutylphthalate
10	19.026	91	204	C ₁₅ H ₂₄	91	204 187 119 105 79	valencene

on GS-Mass.

Extraction and isolation – The dried stem barks (100 g) were refluxed with MeOH for 3 hrs/2 times. The total filtrate was concentrated to dryness *in vacuo* to obtain the MeOH ex. 5g, which was chromatographed on Silica-gel using CHCl₃-MeOH-H₂O (10:1:0→8:2:0.2) to give three fractions (A, B, C). From fraction B, compound **1** was obtained; fraction C afforded compound **2**.

Syringin (1): white needle crystal, C₁₇H₂₄O₉, mp 189~190°. UV λ_{max} (EtOH) 265nm; IR ν_{max}^{KBr} cm⁻¹: 3320, 165, 1595, 1510 and 980; EI-MS(m/z): 372, 210, 182, 167, 154, 149. ¹H-NMR(500 MHz, in MeOD)δppm: 3.20~3.78(6H, m, glucose-H), 3.85(6H, s, 2×-OCH₃), 4.20(2H, dd, J=1.3Hz, 5.6Hz, H-9), 6.35, 6.55(each 1H, m, H-7 and H-8), 6.74(2H, s, H-2 and H-6). ¹³C-NMR(500 MHz, in MeOD)δppm: 135.2(C-1), 105.4(C-2 and C-6), 154.3(C-3 and C-5), 135.9(C-4), 130.0(C-7), 131.3(C-8), 63.6(C-9), glucose-carbon: 105.3(C-1'), 75.7(C-2'), 77.8(C-3'), 71.3(C-4'), 78.4(C-5'), 62.6(C-6'), 57.0(-OCH₃)

β-sitosterol (2): white platelet crystal, C₂₉H₅₀O, mp. 143~145°. EI-MS (m/z): 414, 396, 329, 255, 145, 107.

Results and Discussion

Analyzed the GC/MS results, it is known that the essential oils of the stem barks of AGS are composed of verbenone as main constituent, others including monoterpenes, sesquiterpenes and phthalide groups: *p*-mentha-1,5,8-triene, *n*-butyl isobutylphthalate, *p*-mentha-1,5-diene-8-ol, 8-hydroxy-*p*-cymene, myrtenol, trans-(+)-carveol, 1,3-di-tert-butylbenzene, 4-methyl-2,6-di-butylphenol, and valencene which are similar to those of the root barks of AGS (LIU, X. Q., *et al.*, 2001). They are one of evidences on the herbal medication of the stem barks instead of root barks. The result was in Table 1.

Compound **1** is white needle crystal with molecular formula C₁₇H₂₄O₉, mp 189~190°C and is shown to be

identical with syringin by its spectral data; It is widely distributed in the plant kingdom (CHUNG, B. S., *et al.* 1986).

Compound **2** is white platelet crystal with molecular formula C₂₉H₅₀O, mp. 143~145°C, which was identified by co-IR spectra with an authentic sample.

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