Chemical constituents of Acanthopanax gracilistylus

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Abstract: **Object** To study the chemical constituents of *Acanthopanax gracilistylus* W. W. Smith as the purpose of study continually on plants of *Acanthopanax* (Decne et Planch.) Miq. **Methods** The leaves, roots, and stem barks of *A. gracilistylus* were extracted with hot MeOH and steam distillation respectively, and then, separated and purified by column chromatographies on Diaion HP-20P, Chromatorex ODS, Sephadex LH-20 and silica gel. All compounds were identified on the basis of chemical and spectral analysis including GC-MS, 1D and 2D NMR, MS and IR, or comparison with the reported data. **Results** Six compounds were obtained from the roots of *A. gracilistylus*. They are (-)-pimara-9 (11), 15-dien-19-oic acid (Ⅰ), (-)-kaur-16-en-19-oic acid (Ⅱ), *d*-sesamin (Ⅲ), stigmasterol (Ⅳ), β-sitosterol (Ⅴ), and eleutheroside B (Ⅵ). **Conclusion** Compounds Ⅰ, Ⅳ, and Ⅴ are obtained from this plant for the first time.

Key words: *Acanthopanax gracilistylus* W. W. Smith; Araliaceae; chemical constituent

细柱五加皮化学成分的研究

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摘 要: **目的** 系统地研究五加属植物细柱五加皮 *Acanthopanax gracilistylus* 的化学成分。**方法** 用热甲醇分别对细柱五加皮的根进行提取后, 采用 Diaion HP-20P, Chromatorex ODS, Sephadex LH-20 和硅胶色谱进行分离纯化, 通过光谱分析以及直接和标准品对照进行结构确认。**结果** 从根皮中得到二萜类等 6 个化合物: 五加酸(Ⅰ), 异贝壳杉烯酸(Ⅱ), *l*-芝麻素(Ⅲ), 豆甾醇(Ⅳ), β-谷甾醇(Ⅴ)和刺五加苷 B(Ⅵ)。**结论** 化合物Ⅰ, Ⅳ, Ⅴ为首次从该植物中分离得到。

关键词: 细柱五加皮; 五加科; 化学成分

中图分类号: R284.1 **文献标识码**: A **文章编号**: 0253-2670(2004)03-0250-03

1 Introduction

Acanthopanax gracilistylus W. W. Smith belongs to *Acanthopanax* (Decne et Planch.) Miq. (Araliaceae), has been used as a traditional oriental medicine with tonic, antirheumatic, longitudinal bone growth, adaptogenic activity, fatigue, antitumor activity and gastric ulcer and prophylactic functions for chronic bronchitis hypertension, antistress, ischemic heart disease[1-4]. As an endemic Asian genus, over 35 species have been distributed mainly northeastern Asia including China, Japan, Korea, and in other regions, such as Bhutan, India, Mongolia, Malaysia, Nepal, Philippines, Russia, Thailand, Vietnam, *etc*. Some of them were also found[5, 6]. There are over 26 kinds of plants of *Acanthopanax* (Deche et Planch.) Miq. in China including *A. gracilistylus*, which has been listed in the *China Pharmacopoeia* as *Cortex Acanthopanacis* (named as Wujiapi)[7]. Some lignans and diterpene derivatives from the root and

* 收稿日期: 2003-08-12

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stem bark of *A. gracilistylus* have been isolated and identified[8]. For the purpose of developing and utilizing *Acanthopanax* species resource growing in Asia, the chemical constituents of *A. gracilistylus* collected in Changsha, Hunan Province of China have been systemically investigated. As a result, eight lupane-triterpenoids including three new compounds were obtained from its hot MeOH extracts and seven compounds of essential oils from the leaves, four compounds from the MeOH extracts and ten compounds of essential oils from the stem bark in our previous papers[9-12]. In this manu-script, the isolation and identification of six compounds obtained from the root of *A. gracilistylus* are reported, they are (-)-pimara-9 (11), 15-dien-19-oic acid (Ⅰ)[13], (-)-kaur-16-en-19-oic acid (Ⅱ)[14], *d*-sesamin (Ⅲ)[15], stigmasterol (Ⅳ)[16], β-sitosterol (Ⅴ)[17], and eleutheroside B (Ⅵ)[13], respectively. Among them, compounds Ⅰ, Ⅳ, and Ⅴ are obtained for the first time from this plant.

2 Experiment

2.1 Plant materials. The samples of *A. gracilistylus* were collected in March 2001 in Changsha, Hunan Province of China, and identified by Prof. YOOK Chang-soo, College of Pharmacy, Kyung Hee University in Korea. A voucher specimen has been deposited in the Pharmacognosy Laboratory, College of Pharmacy, Kyung Hee University in Korea.

2.2 General. Melting points (uncorrected) were measured on a Boetius micromelting point apparatus. Optical rotations were determined on a JASCO DIP-1000KUY polarimeter (l = 0.5). IR spectra were obtained with a Hitachi 270-30 type spectrophotometer, and NMR spectra were measured in methanol-d_4 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), Diaion HP-20P (Mitsubishi Chem. Ind. Co., Ltd., Japan), Chromatorex ODS (30-50 μm, Fuji Silysia Chem. Ind. Co. Ltd., Japan) and Sephadex LH-20 (Pharmacia Biotech, Sweden). TLC was performed on precoated silica gel 60 GF 254 (Merck) and RP-18F_{254S} (Merck) plates.

2.3 Extraction and isolation. The powder of air-dried root barks were extracted with hot MeOH two times, and then separated with ether, 22.4 g of dried extract was obtained. From them, 10 g extracts was chromatographed on silica gel CC using *n*-hexane-EtOAc (10∶1→2∶1), and four fractions (Fr. A, B, C, and D) were obtained. Fraction A was chromatographed on silica gel CC eluting with *n*-hexane-EtOAc (10∶1→5∶1), compounds Ⅰ (100 mg) and Ⅱ (20 mg) were obtained. Fraction B afforded compound Ⅲ (10 mg) by recrystalization. Fraction C afforded compound Ⅳ (15 mg) by recrystalization. Fraction D eluted with hexane-EtOAc (40∶1) on silica gel column afforded compound Ⅴ (20 mg) and compound Ⅵ (16 mg).

3 Identification

Compound Ⅰ [(-)-pimara-9 (11), 15-dien-19-oic acid]: $C_{20}H_{30}O_2$, amorphous powder, mp 135 ℃- 136 ℃, IR ν_{max}^{KBr} (cm^{-1}): 3 290 (OH), 1 690 (C=O), 1 638 (C=C), 1 460, 1 075, 965. ^{1}H-NMR (500 MHz, CD_3OD) δ 1.03 (3H, s, H-20), 1.07 (3H, s, H-17), 1.18 (3H, s, H-18), 4.86 (1H, dd, J = 10.3, and 1.4 Hz, H-16a), 4.98 (1H, dd, J = 17.5, and 1.3 Hz, H-16b), 5.41 (1H, m, H-11), and 5.84 (1H, dd, J = 17.5, and 10.8 Hz, H-15). ^{13}C-NMR (125 MHz, CD_3OD) δ 43.24 t (C-1), 20.28 (C-2), 38.70 (C-3), 45.16 (C-4), 49.09 (C-5), 21.62 (C-6), 29.19 (C-7), 29.96 (C-8), 151.38 (C-9), 39.65 (C-10), 117.72 (C-11), 39.57 (C-12), 35.91 (C-13), 43.30 (C-14), 151.51 (C-15), 109.73 (C-16), 22.76 (C-17), 29.24 (C-18), 181.68 (C-19), 23.10 (C-20).

Compound Ⅱ [(-)-kaur-16-en-19-oic acid]: $C_{20}H_{30}O_2$, needle crystals, mp 179 ℃- 180 ℃, IR ν_{max}^{KBr} (cm^{-1}): 3 420 (OH), 1 690 (C=O), 1 655, and 875 (C=C). ^{1}H-NMR (500 MHz, CD_3OD) δ 0.97 (3H, s, H-20), 1.18 (3H, s, H-18), 2.61 (1H, m, H-13), 4.72 (1H, brs, H-17a) and 4.78 (1H, brs, H-17b). ^{13}C-NMR (125 MHz, CD_3OD) δ 42.10 (C-1), 20.37 (C-2), 39.24 (C-3), 44.68

(C-4), 58.29 (C-5), 23.13 (C-6), 42.59 (C-7), 45.45 (C-8), 56.56 (C-9), 40.81 (C-10), 19.50 (C-11), 34.26 (C-12), 45.26 (C-13), 40.84 (C-14), 50.18 (C-15), 156.77 (C-16), 103.75 (C-17), 29.56 (C-18), 181.68 (C-19), 16.39 (C-20).

Compound Ⅲ (*d*-sesamin): $C_{20}H_{18}O_6$, colorless prisms, mp 123 ℃- 125 ℃, $[\alpha]_D$ - 68 °(*c* = 1.0 in $CHCl_3$), IR ν_{max}^{KBr} (cm^{-1}): 2 969, 2 942, 2 904 (aromatic CH), 2 825 (aliphatic CH), 1 500, 1 444 (aromatic C = C), 1 195 (aromatic-C-O), 927 (methyl-ene oxime). R_f value was in agreement with its authentic sample.

Compound Ⅳ (stigmasterol): $C_{29}H_{48}O$, colorless needles, mp 168 ℃- 169 ℃. ^{1}H-NMR (500 MHz, in CD_3OD) δ 0.73 (s, 3H, H-18), 0.88, 0.93 (each, 3H, H-26, and 27), 0.97 (s, 3H, H-29), 1.04 (s, 3H, H-19), 1.84 (s, 3H, H-21), 3.34 (m, H-3), 4.95 (1H, m, H-23), 5.19 (1H, m, H-22), 5.34 (1H, d, *J* = 5.2 Hz, H-6). ^{13}C-NMR (125 MHz, in CD_3OD) δ 142.23, 138.51, 130.60, 122.44, 72.44, 57.46, 57.37, 52.80, 51.73, 41.92, 41.15, 41.03, 38.55, 37.43, 35.10, 32.29, 30.37, 30.18, 29.35, 25.31, 24.14, 21.76, 21.54, 20.18, 19.85, 19.40, 19.31, 12.33, 12.30.

Compound Ⅴ (β-sitosterol): $C_{29}H_{50}O$, needle-like crystals, mp 138 ℃- 140 ℃. EI-MS (rel. int. %): 414 (100) $[M]^+$, 396 (45), 329 (27), 303 (30), 255 (15), 145 (20), 107 (25), 95 (20).

Compound Ⅵ (eleutheroside B): $C_{17}H_{24}O_9$, white needle crystals, mp 189 ℃- 190 ℃. ^{1}H-NMR (500 MHz, in CD_3OD) δ 3.20-3.78 (6H, m, glucose-H), 3.85 (6H, brs, 2 × OCH_3), 4.20 (2H, dd, *J* = 1.3 Hz, 5.6 Hz, H-9), 6.35, 6.55 (each 1H, m, H-7, and H-8), 6.74 (2H, s, H-2, and H-6). ^{13}C-NMR (125 MHz, in CD_3OD) δ 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 (-OCH_3).

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