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Studies on the Chemical Constituents of *Acanthopanax koreanum* (II)

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Abstract □ From the root bark of *Acanthopanax koreanum,* two polyacetylene compounds and one lignan compound were isolated and identified as falcarinol, falcarindiol and ariensin. Furthermore the stem bark also afforded methyl n-hexacosanoate, methyl linoleate and coniferin.

Keywords □ *Acanthopanax koreanum,* Araliaceae, falcarinol, falcarindiol, ariensin, methyl n-hexacosanoate, methyl-linoleate, coniferin.

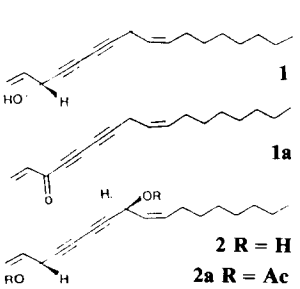
The root and stem bark of *Acanthopanax koreanum* NAKAI (Araliaceae) is widely used as a herbal remedy for rheumatism, tonic, paralysis and sedative agents.[1] It has reported that earlier investigations on this plant showed lignan[2] and phenylpropanoid compound.[3] Further studies on the root and stem bark of this plant have resulted polyacetylenes, lignan, fatty acid methylesters and phenylpropanoid compound. The ether soluble fraction from the MeOH extract of the root bark was chromatographed repeatedly with silica gel column and gave compounds **1, 2** and **3.**

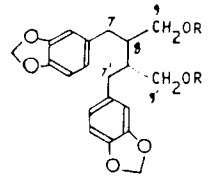
Compound **1,** yellow-like oil, showed characteristic UV absorption[4] of polyacetylene (229.3, 240.3, 254.0 and 280.7 nm) and conjugated diacetylenes directly bonded to the -CHOH group (2,260 cm^{-1}) and typical conjugated *cis* C = C double bond (695 cm^{-1}) in its IR spectra.[5] ^{1}H-NMR spectra exhibited terminal vinyl group (δ 5.09-5.94), protons of internal double bond (δ 5.16-5.51) and allylic protons to terminal vinyl group (δ 4.87). The downfield shift of doublet proton at δ 4.87 indicated that the hydroxyl group was between double bond and triple bond.[6] The mass spectra showed small molecular ion peak at *m/z* 244 and [M^+-C_6H_{13}] peak by allylic fission at *m/z* 159, indicating that it had aliphatic C_7H_{15} moiety and 3-hydroxy hept-1-ene-4, 6-diyne moiety in this compound.[7] All the chemical and spectral data including ^{13}C-NMR were identical with falcarinol which was reported in literature.[8] Compound **1** was converted to dark brown substances by preservation in the room temperature for a long time and it was identified falcarinone(**1a**) by ^{1}H-NMR spectra.[9]

Compound **2,** viscous yellow-like oil, showed similar spectral pattern with that of compound **1,** and characterized as falcarindiol by analysis of the UV, IR, MS, ^{1}H-NMR and ^{13}C-NMR spectra of **2** and **2a.**[10,11]

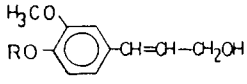
Compound **3** is a colourless viscous liquid, showing aromatic ring absorption at λ max (EtOH) 287 nm is the UV spectra and ester carbonyl absorption at 1730 cm^{-1} in the IR spectra. The apparent odd number of protons displayed in the ^{1}H-NMR spectra showed the dimeric nature of the molecule, in addition to a methylenedioxy group at δ 5.91, there are one acetate singlet at δ 2.05, three aromatic protons at δ 6.51-6.68, one methylene of an esterified primary alcohol at δ 4.03, one benzylic methylene at δ 2.60 and one methine proton at δ 2.10.[12] These data showed that compound **3** was a neolignan belonged to the group of austrobailignans. In the MS spectra the molecular ion peak appeared at *m/z* 442 and fragmentation pattern showed that compound **3** was symmetrical configuration. Hydrolysis of compound **3** gave the corresponding solid diol **3a,** mp, 79-81 °C. Its spectral data, given in the experimental methods, were in full agreement with those corresponding to its structure.[13] Compound **1, 2** and **3** were first isolated in Acanthopanax species.

The ether and BuOH extract of stem bark was chromatographed with silica gel repeatedly and gave two fattyacid methylesters and one phenyl-





3 R = Ac
3a R = H



6 R = Glucose
6a R = H

H_3CO-CO-CH_2-$(CH_2)_{20}$-CH_2-CH_2-CH_3 **4**

H_3CO-CO-$(CH_2)_7$-CH=CH-CH_2-CH=CH-$(CH_2)_4$-CH_3 **5**

propanoid compound respectively.

Compound **4** was crystallized from acetone to give white needle crystals, mp 58-59 °C. The IR spectra of this compound indicated the presence of ester carbonyl at 1742 cm^{-1}. The ^{1}H-NMR spectra showed methyl signal at δ 0.88 (3H, brt), long chain methylene protons at δ 1.28, methylene protons adjacent to ester carbonyl at δ 2.3 and methoxyl group at δ 3.68.

It showed an molecular ion peak at m/z 410, other fragment peaks at m/z 396, 382, 143 and 57. From these spectral data compound **4** was identified as methyl n-hexacosanoate.

Compound **5**, colourless oil, showed ester absorption at 1745 cm^{-1} in IR spectra and methyl, methylene, methoxy and double bond protons at 1.28, 1.31, 3.68 and 5.35 in the ^{1}H-NMR spectra respectively. MS spectra showed characteristic fragments pattern of fattyacid methylester (m/z 294 [M^+], 269, 149, 95, 83, 71, 69). Comparisons of ^{13}C-NMR data between various unsaturated fatty-acid methylester allowed us to assign all signals to the methyl, methylene and methoxy carbonyl group.[14,15] In this comparison compound **5** was assigned to the methyl linoleate.

Compound **6** was crystallized from the mixture of acetone and MeOH and gave white needle crystals, mp 188-190 °C, $C_{16}H_{22}O_8$ (C: 54.42%, H: 6.23%). The ^{1}H-NMR spectra showed methoxy group (δ 3.76), vinylic proton (δ 6.36) and aromatic proton (δ 7.07).[16] We obtained a small parent peak at m/z 342 and a cleavege with hydrogen transfer to give m/z 180 [$C_{10}H_{12}O_3^+$] for the aglycone in the MS spectra. The identify of this compound was based on the comparison of ^{13}C-NMR spectral data[17] and identified as coniferin.

EXPERIMENTAL METHODS

Plant materials

The root and stem barks of Acanthopanax koreanum was collected at the Hanla Mt. of Korea in 1983.

General procedures

Acetylation of a compound was performed with AC_2O/pyridine at room temperature and methylation was carried out with diazomethane in the usual way. Mps were determined Yanagimoto micro-melting point apparatus and were uncorrected. IR absorption spectra were obtained on a Beckmann IR-20A spectrophotometer and Jasco Model 701G spectrophotometer. A recording spectrophotometer, LKB(Biochrom) Ultrospec 4050 UV spectrophotometer was used for the measurements of UV absorption spectra. NMR spectra were taken at 25 °C using tetramethylsilane (TMS) as an internal standard on a Varian Model FT 80A spectrometer (80 MHz) or Jeol JMN FX-100 NMR spectrometer (100 MHz). El-MS spectra were obtained on a Hewlett-Packard GC/MS spectrometer (type 5985 B) or Jeol JMS-DX 300.

Extraction and isolation of compounds

Dried root barks (8kg) and stem barks (2kg) were crushed and extracted with MeOH. The MeOH extracts were concentrated to dryness and then partitioned into ethylether and n-BuOH. The ethylether extact (350g) of root barks was separated on silica gel with hexane-ethylacetate (20:1→5:1) as eluant. The following three compounds were isolated.

Falcarinol (1)

Yellow-like oil. UV λ max in hexane (nm): 229.3, 240.3, 254.0, 280.7; IR (cm^{-1}): 3350(-OH), 2260, 2240 (-C≡C-), 1645, 985, 932 (-C≡C-), 695; MS m/z (rel. int. %): 244 (M^+, 0.6), 159 (39.6), 131 (44.9), 117 (60.4), 115 (73.8), 91 (98.1), 57 (15.3), 55 (76.9), 43 (71.7); ^{1}H-NMR (80MHz, $CDCl_3$) δ: 0.88 (3H, s, H-17), 1.27 (1OH, m, H-12, 13, 14, 15, 16), 2.01 (2H, m, H-11), 3.00 (2H, d, J=7.0 Hz, H-8), 4.87 (1H, d, J=5.3 Hz, H-3), 5.09-5.51 (4H, m, H-1, 9, 10), 5.94 (1H, m, H-2); ^{13}C-NMR: see Table I.

Falcarinone (1a)

^{1}H-NMR (80 MHz, $CDCl_3$) : 0.88 (3H, t, -CH_3), 1.26 (10H, m, 12, 13, 14, 15, 16, -H), 2.04 (2H, m, 11-H), 3.13 (2H, d, J=5.5 Hz 8-H), 5.43-5.52 (2H, m, 9, 10-H), 6.13-6.52 (3H, m, 1,

Table I. ^{13}C-NMR data for 1, 2 and 2a ($CDCl_3$, 90 MHz)

carbon	1	2	2 a
1	116.43	116.98	119.5
2	136.12	135.61	136.3
3	63.02	63.03	64.2
4	79.57	79.60	76.7
5	70.81	70.00	69.2
6	64.13	68.49	65.1
7	74.40	78.12	75.0
8	17.42	58.15	60.0
9	121.93	127.48	123.7
10	132.64	134.02	131.9
11	26.93	27.48	27.7
12	31.59	31.61	31.7
13	28.94	28.96	29.0
14	28.94	28.96	29.0
15	28.94	29.10	29.0
16	22.40	22.46	22.5
17	13.79	13.92	13.9
-$OCOCH_3$			169.2, 169.5
-$OCOCH_3$			20.6

2-H).

***Falcarindiol* (2)**

Colorless viscid oil. UV δ max in ethylether (nm): 232.6, 253.4, 265.3, 284.0; IR (cm^{-1}): 3365 (-OH), 2250, 2115 (-C C-), 1650 (-C C-) MS *m/z* (rel. int. %): 260 (M^+, 0.3), 185 (3.6), 175 (4.7), 157 (35.6), 143 (24.5), 129 (100.0), 115 (71.1), 105 (38.5), 91 (78.2); ^{1}H-NMR (200 MHz, $CDCl_3$) δ : 0.90 (3H, t, J = 6.5 Hz, H-17), 1.26 (10H, brs, H-12, 13, 14, 15, 16), 2.10 (2H, m, J = 7.5 Hz H-11), 3.54 (2H, brs, -OH), 4.92 (1H, d, J = 5.5 Hz H-2); ^{13}C-NMR: see Table I.

***Falcarindiol acetate* (2a)**

Colorless viscid oil. UV λ max in ethylether (nm): 232.0, 245.0, 258.5; IR (cm^{-1}): 2145 (-C C-), 1740 (-C = O), 1640 (-C = C-) MS (rel. int. %) *m/z*: 295 (6.0), 233 (17.3), 175 (9.4), 161 (21.9), 115 (19.4); ^{1}H-NMR (80 MHz, $CDCl_3$) δ : 1.27 (3H, $-CH_3$), 2.06 (3H, -OAc), 2.08 (3H, -OAc), 5.25-6.10 (7H, m, H-3, 8, olef. H); ^{13}C-NMR: see Table I.

***Ariensin* (3)**

Colorless oil. UV max in EtOH (nm): 287; IR (cm^{-1}): 1730 (-C = O), 1610, 1043, 930; MS *m/z* (rel. int. %): 442 (M^+, 11.7), 382 (1.1), 220 (2.3), 187 (20.1), 174 (8.0), 148 (4.9); ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 2.05 (6H, s, 2x-OAc), 2.10 (2H, m, H-8, 8′), 2.60 (4H, m, H-7, 7′), 4.03 (4H, m, H-9, 9′), 5.91 (4H, s, 2x-O-CH_2-O-), 6.51-6.68 (6H, m, H-Ar);

***Hydrolysis of ariensin* (3a)**

A solution of 80 mg of 3 in 10 m*l* of MeOH was mixed with a solution of 1 g of KOH in 1 m*l* of H_2O and 10 m*l* of MeOH. The mixture was refluxed for 6hr, concentrated, poured over ice water and extracted with ethylacetate. The ethylacetate layer was washed with water, dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was chromatographed over 15 g of silica gel and eluted hexane-ethylacetate (8:1). The crystalline fractions were combined and recrystallized from chloroform-hexane, yielding 50 mg of white crystals (3a), mp, 79-81 °C, IR (cm^{-1}, KBr): 3040 (-OH), 1045, 930; ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.85 (2H, m, 8, 8′-H), 2.67 (4H, m, 7, 7′-H), 3.63 (4H, m, 9, 9′-H), 5.85 (4H, s, 2x-O-CH_2-O-), 6.52-6.70 (6H, m, Ar. -H); MS *m/z* (rel. int. %): 358 (M^+ 4.8), 187 (5.4), 173 (2.6), 161 (3.2), 149 (2.8), 135 (100.0).

***Methyl n-hexacosanoate* (4)**

White needle crystals, mp: 58-59°; IR (cm^{-1}, KBr): 1742 (C = O); ^{1}H-NMR (100 MHz, $CDCl_3$) δ ; 0.88 (3H, t, $-CH_3$), 1.28 (4OH, -$(CH_2)_{20}$-), 2.3 (2H, -CO-CH_2-), 3.68 (3H, -OCH_3); ^{13}C-NMR (25 MHz, $CDCl_3$) δ : 173.81 (C = O), 51.45 (-OCH_3), 34.05 (C-2), 31.94 (C-24), 29.71 (C-4-23), 24.96 (C-3), 22.68 (C-25), 14.06 (-CH_3); MS *m/z* (rel. int. %): 410 (M^+, 61.2), 396 (11.1), 382 (20.5), 143 (22.3), 87 (71.8), 74 (100.0), 57 (47.0).

***Methyl linoleate* (5)**

Colourless oil, IR (cm^{-1}): 1745 (C = O); ^{1}H-NMR (100 MHz, $CDCl_3$) δ : 0.90 (-CH_3), 1.28, 1.31 (-CH_2-), 3.68 (-OCH_3), 5.35 (=CH-); ^{13}C-NMR (25 MHz, $CDCl_3$) δ : 174.10 (C-1), 34.10 (C-2), 25.05 (C-3), 29.30 (C-4, 5, 6), 29.75 (C-7), 27.35 (C-8), 130.05 (C-9), 128.25 (C-10), 25.75 (C-11), 128.10 (C-12), 130.20 (C-13), 27.35 (C-14), 29.45 (C-15), 31.70 (C-16), 22.70 (C-17), 14.10 (C-18), 51.30 (-OCH_3); MS *m/z* (rel. int. %): 294 (M^+, 18.0), 263 (M^+-CH_3OH, 11.2), 149 (36.04), 95 (45.7), 83 (51.4), 71 (60.7), 69 (97.5), 57 (94.9), 55 (100.0).

***Conferin* (6)**

White needle crystal, mp: 188-190 °C; IR (cm^{-1},

KBr): 3380 (-OH), 1587, 1512; ^{1}H-NMR (100 MHz, DMSO-d_6) δ : 3.76 (-OCH_3), 4.16 (-CH_2), 6.36 (-CH=CH-), 7.07 (3H, Ar. -H); ^{13}C-NMR (25 MHz, DMSO-d_6) δ ; 130.81 (C-1), 109.82 (C-2), 148.73 (C-3), 145.68 (C-4), 115.21 (C-5), 118.67 (C-6), 128.69 (C-7), 128.10 (C-8), 61.41 (C-9), 55.55 (-OCH_3), 99.97 (C-1 '), 74.22 (C-2 '), 76.65 (C-3 '), 69.50 (C-4 '), 76.77 (C-5 '), 60.53 (C-6 '); MS *m/z* (rel. int. %): 342 (M^+, 0.6), 180 (M^+, -glucose, 100.0), 137 (36.8), 124 (22.5).

Enzyme hydrolysis of coniferin **(6a)**

Coniferin (30 mg) was hydrolyzed with -glucosidase (10 mg, Sigma) in H_2O for 1 day at 37 °C. The reaction mixture was extracted with Et_2O and the residue obtained from the Et_2O layer was chromatographed on silica gel. Elution with $CHCl_3$-MeOH (10:1) gave a pure aglycone (6a). ^{1}H-NMR (100 MHz, $CDCl_3$) δ : 3.90 (3H, s, -OCH_3), 4.26, 4.31 (2H, d, -CH_2-), 6.10-6.65 (-CH=CH-), 6.88 (3H, m, Ar. -H); MS *m/z* (rel. int. %): 180 (M^+, 21.79), 137 (31.27), 86 (63.04), 84 (100.0).

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