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A Lignan from *Rubia akane*

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Abstract□A lignan, (+)-isolariciresinol, was isolated from *Rubia akane* Nakai. This is the first reporting from Rubiaceae.

Keywords□*Rubia akane*, Rubiaceae, lignan, (+)-isolariciresinol.

Rubia akane Nakai (Rubiaceae) has been used for hemostatic, jaundice, bronchitis and hematemesis[1]. Anthraquinones[2-5], including alizarin, purpurin and pseudopurpurin, and cyclohexapeptide alkaloids, RA-V and RA-VII[6-12], which have antineoplastic activity, were reported from *R. akane*. In this study, we report a lignan, (+)-isolariciresinol from *R. akane*. This is the first occurrence of (+)-isolariciresinol in Rubiaceae.

EXPERIMENTAL

Melting point was uncorrected. IR spectra were recorded on KBr disc (Perkin-Elmer 281B Spectrometer). NMR spectra were obtained on either Varian VXR-200S Spectrometer (200 MHz) or Bruker AM-300 WB Spectrometer (300 MHz) using TMS as an internal standard. ElMS were determined on Hewlett-Packard 5985B GC/MS System equipped with direct inlet system.

Plant material

The whole plant of *R. akane* (14 kg) was collected from Kyeong-Ki Do, Korea in 1988. The plant was verified by Dr. Seung-Jo Yoo, Professor, College of Pharmacy, Sung Kyun Kwan University, Suwon, Korea.

Extraction and isolation

Air dried plant was extracted with methanol (20 $l \times 3$). After removal of solvent, MeOH extract was dissolved in H_2O (4 l) and fractionated with Et_2O (4 $l \times 3$). Et_2O layer was extracted with 5% HCl (4 $l \times 3$), neutralized with c-NH_4OH, and extracted with $CHCl_3$ (6 $l \times 3$). $CHCl_3$ layer was evaporated in vacuo and dried with anhydrous Na_2SO_4.

$CHCl_3$ extract (16g) was adsorbed on silica gel (500g). Elution was commenced with $CHCl_3$ and continued stepwise through $CHCl_3$-MeOH mixtures (40:1), produced eight main groups, Fr.1-6 (11g), Fr.7 (2.4g) and Fr.8 (560 mg). Fraction 7 (2.4g) was adsorbed on silica gel (35g), eluting with n-Hexane-EtOAc mixtures (10:1-0:100), yield five fractions, Fr.7-1 to 2 (355 mg), Fr.7-3 (790 mg), and Fr.7-4 to 5 (530 mg). Fraction 7-3 (790 mg) was purified by silica gel column chromatography (silica gel 12g) eluting with EtOAc, yield five fractions (7-3-1 to 7-3-5). Fraction 7-3-3 (710 mg) was recrystallized from $CHCl_3$-MeOH (20:1), gave Compound A (110 mg), white needle, $C_{20}H_{24}O_6$. $[\alpha]_D$ +53.5 (c 1.0, Me_2CO), mp 153-154°C (lit. [15] 158-160°C or 114-115°C from aqueous methanol). IR (cm^{-1}, KBr): ν_{max} 3400-3300 (OH), 1600, 1500 (Arom.) UV (MeOH): λ_{max} nm (log ε) 220 (4.23), 285 (3.90) Mass [E.I. m/z] (Rel. Int. %): 360 (95.8, M^+), 342 (8.0), 325 (4.6), 311 (31.3), 296 (9.2), 284 (55.7), 279 (35.1), 271 (35.9), 255 (43.6), 241 (83.0), 225 (14.1), 211 (26.5), 197 (26.0), 187 (59.3), 175 (100.0), 137 (76.3), 115 (31.7). ^{1}H-NMR (300 MHz, CD_3OD): (Table I) ^{13}C-NMR (50 MHz, CD_3OD): (Table II).

Methylation of compound A

Compound A (3 mg) in methanol (200 μl) was treated with 500 μl of diazomethane solution in ether at room temperature for 1 hr. After removal of solvent, the reaction product was purified by silica gel column chromatography (silica gel 1g) eluting with $CHCl_3$-MeOH mixtures (50:1) to obtain Compound A-dimethyl ether. Mass [E.I. m/z] (Rel. Int. %): 388 (100.0), 370 (7.3), 355 (4.3), 339 (20.8), 269 (63.8), 238 (23.1), 189 (39.9), 165 (17.2), 151 (944.7).

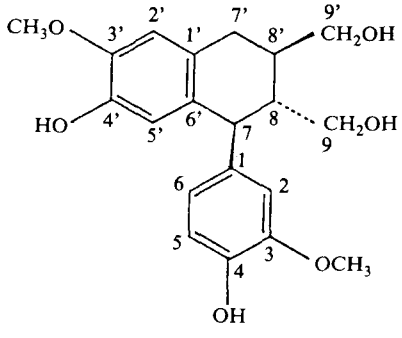
Acetylation of compound A

Compound A (3 mg) was dissolved in 200 μl of trifluoroacetic anhydride and 100 μl of acetic acid, and stood at room temperature for 30 mins. The reac-

Table I. ^{1}H-NMR spectral data of Compound A, comparison with lit. [15], and ent-isolariciresinol (lit. [14])

H	Compound A (CD_3OD)	(+)-isolariciresinol (CD_3OD)	ent-isolariciresinol (CD_3OD)
2	6.68d (1.9)*	6.69d (2.0)	6.60d (1.98)
5	6.74d (8.0)	6.84d (8.0)	6.66d (7.9)
6	6.61dd (8.0, 1.9)	6.58dd (8.0, 2.0)	6.52dd (7.9, 1.98)
7			
8	1.76m	1.62-2.10m	1.69m
9a, b	3.68m		3.21m
	3.40dd (11.2, 4.1)		
2'	6.24s	6.28s	6.11d (0.79)
5'	6.65s	6.67s	6.54d (0.79)
7'	2.77d (7.7)	2.91m	2.68d (7.30)
8'	2.01m	1.62-2.10m	1.91m
9'	3.68m		3.31m
OMe	3.80s	3.85s	3.65s
OMe	3.77s	3.82s	3.67s

*J(Hz) in parentheses



tion mixture was neutralized and then extracted with $CHCl_3$. $CHCl_3$ extract was purified by preparative TLC developing with $CHCl_3$-MeOH mixtures (200:1) to obtain Compound A-tetraacetate. Mass [E.I. m/z] (Rel. Int. %): 528 (2.5, M^+), 486 (13.5), 444 (25.2), 426 (24.7), 402 (7.0), 784 (25.2), 311 (100.0), 284 (36.0), 241 (10.9), 137 (22.8).

RESULTS AND DISCUSSION

The chloroform extract of the whole plant of *R. akane* was prepared as described in the experimental section and was purified by silicagel column chromatography to obtain Compound A.

Table II. ^{13}C-NMR spectral data of Compound A, comparison with lit. [13], and ent-isolariciresinol (lit. [14]

C	Compound A (CD_3OD)	(+)-isolaricire-sinol (CD_3OD)	ent-isolaricire-sinol (CD_3OD)
1	134.1	132.6	134.1
2	113.7	112.0	114.0
3	147.1	145.2	147.1
4	145.2	143.5	145.1
5	115.9	114.5	116.0
6	123.2	121.9	123.1
7	48.0	47.4	48.0
8	48.0	47.5	48.0
9	62.2	62.1	62.5
1'	128.9	127.2	129.0
2'	112.3	110.6	112.4
3'	149.0	147.1	148.9
4'	145.9	144.1	145.8
5'	117.3	115.8	117.3
6'	138.6	136.8	138.5
7'	33.6	32.8	33.5
8'	40.0	39.5	40.1
9'	65.9	65.7	66.0
OMe	56.4	55.6	56.4
OMe	56.3	55.6	56.4

Compound A was recrystallized from chloroform-methanol, molecular formular $C_{20}H_{24}O_6$ [M^+, 360]. IR absorption peaks at 3400 cm^{-1} and 1600-1500 cm^{-1} suggested the presence of hydroxyl groups and aromatic rings, respectively.

Bathochromic effect in UV spectrum with an addition of NaOMe suggested the presence of phenolic $-$OHs. Compound A produced dimethyl ether (M^+, 388) by methylation, and tetraacetate (M^+, 528) by acetylation. The ^{13}C-NMR spectra reveal signals for 20 carbons; two CH_3, three CH_2, eight CH (five sp^2) and seven completely substituted carbons (six sp^2). The ^{1}H-NMR spectrum shows signals of two OMes, and two CH-CH_2OH. One of aromatic systems was found to be 1,3,4-trisubstituted, the other was 1,2,4,5-tetrasubstituted. From ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectra, a doublet at 2.77 ppm of CH_2 protons suggested Ar-CH_2-CH.

Comparison of the spectral data of Compound A with those published for (+)-isolariciresinol and ent-

isolariciresinol established its identity. Those ^{1}H-NMR and ^{13}C-NMR data were listed (Table I and II). Compound A has $[\alpha]_D+53.3$ (c 1.0, Me_2CO) and $[\alpha]_D+39.3$ (c 1.0, MeOH), identified as (+)-isolariciresinol which has $[\alpha]_D+68$ (c 1.0, Me_2CO)[13], while ent-isolariciresinol has $[\alpha]_D-53.8$ (c 1.47, MeOH)[14]. (+)-isolariciresinol has been isolated from three plant sources[13, 15, 16], but this is the first reported isolation of (+)-isolariciresinol from Rubiaceae.

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