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Constituents of *Lindera Erythrocarpa* Stem Bark

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Abstract – Three chalcones and a stilbenoid have been isolated from the stem bark of *Lindera erythrocarpa*. They were elucidated as 2'-hydroxy-3',4',5',6'-tetramethoxychalcone, 2',4'-dihydroxy-3',6'-dimethoxy chalcone, 2',4',5',6'-tetrahydroxy-3'-methoxychalcone, and 5,6-dihydroxy-2,3,4-trimethoxystilbene. Chemical structures were elucidated on the basis of MS, ^{1}H, ^{13}C and 2D-NMR spectroscopic data. This is the first report on the isolation of these compounds from the *L. erythrocarpa*.
Keywords – *Lindera erythrocarpa*, stem bark, 2'-hydroxy-3',4',5',6'-tetramethoxychalcone, 2',4'-dihydroxy-3',6'-dimethoxychalcone, 2',4',5',6'-tetrahydroxy-3'-methoxychalcone, 5,6-dihydroxy-2,3,4-trimethoxystilbene

Introduction

Lindera erythrocarpa (Lauraceae) is a deciduous shrub and their twigs are light brown. Its leaves are alternate form, belong to oblancecolate, 6-13 cm long and 1.5-2.5 cm wide, and are light green and glabrous. It is distributed throughout China, Korea and Japan (Sun and Chung 1988). The leaves of this tree have been used as a folk medicine for stomach and thirst (Liu *et al.*, 1973a). From the barks and the leaves of *L. erythrocarpa*, Liu *et al.* (1973a, 1973b) isolated linderone, methyllinderone, lucidone and methyllucidone, and also identified such as sitosterol-D-glucoside. From *L. erythrocarpa*, Komae and Hayashi (1972) also isolated geranyl acetate and caryophyllene. From the leaves of *L. erythrocarpa*, a stereoisomer complex of methyllucidone was isolated (Choi *et al.*, 2004).

In this paper, we described the isolation and structural determination of three chalcones and a stilbenoid from the stem bark of *L. erythrocarpa*.

Materials and Methods

Plant materials – The bark of *L. erythrocarpa* was collected from Kumsan mountain, Kyungnam, Korea, in 1996, and dried at room temperature. The voucher specimens are deposited at the Korea Forest Research Institute, Seoul, Korea. After drying, these samples were ground with a Wiley mill.

Extraction, fractionation and Isolation – Dried and ground bark of *L. erythrocarpa* was extracted twice with ethanol (EtOH) and then evaporated to give the crude extracts. The crude extracts (55.1 g) was successively par titioned with organic solvents, such as *n*-hexane, di chloromethane (CH_2Cl_2) and ethyl acetate (EtOAc) in this order.

To isolate the compounds, silica gel and Sephadex LH-20 were used for column chromatography with eluting solvents. The *n*-hexane soluble fraction (16.8 g) was used for the isolation. This part of extracts was chroma-tographed on silica gel column (65 × 5.0 cm) eluted with *n*-hexane-EtOAc (9:1, v/v) to give 11 sets of fractions (LE-1~LE-11). The LE-5 fraction was further subjected to repeated column (80 × 4.0 cm) chromatography on the Sephadex LH-20 eluted with methanol to give 4 sets of fractions (LE-5-1~LE-5-4). Compound **1** (3.2 g) was obtained from the second fraction (LE-5-2) of LE-5. The LE-4 fraction was further subjected to repeated column (80 × 4.0 cm) chromatography on the Sephadex LH-20 using me-thanolethanol (1:1; m/v) to give 3 sets of fractions (LE-4-1~LE-4-3). Compound **2** was obtained from the first fraction (LE-4-1). The LE-6 fraction was further subjected to column chromatography over silica gel (benzene-CH_2Cl_2, 9:1, v/v) to generate 11 sets of fractions (LE-6-1~LE-6-11). Compound **3** (22.0 mg) was obtained from the fra ction LE-6-6. Repeated column ch-romatography over silica gel with benzene-MeOH (19:1, v/v) of the fraction

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LE-8 (2.0 g) gave 7 fractions (LE-8-1~LE-8-7). Compound **4** (1.6 g) was obtained from the fraction LE-8-6.

Instrumental analysis – EI-MS was performed at 70 eV ionization energy by direct inlet probe method, using JEOL JMS-600W mass spectrometer. ^{1}H-, ^{13}C- and 2D-NMR spectra were obtained using a Varian UI 500 spectrometer at the operating frequency of 500 MHz (^{1}H) and 125 MHz (^{13}C) at the Korea Basic Science Institute in Seoul.

Compound 1 – ^{1}H- and ^{13}C-NMR data (Table 1). ^{1}H-^{1}H COSY correlations : H-β↔H-α, H-2/H-6↔H-3/H-5. HMBC correlations : H-2/6→C-3,5/C-β, H-3,5→C-1, H-β→C-1. EI-MS *m/z* : 344 (M^+, molecular ion), 267, 240, 197, 103.

Compound 2 – ^{1}H- and ^{13}C-NMR data (Table 1). ^{1}H-^{1}H COSY correlations : H-β↔H-α, H-2/H-6↔H-3/H-5. HMBC correlations : H-α→C-1/C-β', H-β,→C-1/C-β'/C-2, 6, H-2, 6→C-4/C-β, H-3, 5→C-2, 6, H-5'→C-1'/C-3'/C-4', H-OCH_3→C-6', H-OCH_3→C-3'. EI-MS *m/z* : 300 (M^+, molecular ion), 257, 223, 181, 153, 103.

Compound 3 – ^{1}H- and ^{13}C-NMR data (Table 2). ^{1}H-^{1}H COSY correlations : H-β↔H-α, H-2/H-6↔H-3/H-5. HMBC correlations : H-α→C-1/C-β', H-β↔C-1/C-β'/C-2, 6, H-2, 6→-4/C-β, H-3, 5→C-2, 6, H-5'→C-1'/C-3'/C-4', H-OCH_3→C-6', H-OCH_3→C-3'.

Compound 4 – ^{1}H- and ^{13}C-NMR data (Table 2). ^{1}H-^{1}H COSY correlations : H-β↔H-α, H-2'/H-6'↔-3'/H-5'. HMBC correlations : H-α→C-1'/C-1, H-2'/6'→C-β, H-β→C-1/C-2'/6', H-3'/5'→C-2'/C-6'/C-1', H-OCH_3→C-4, H-OCH_3→C-2, H-OCH_3→C-3. EI-MS *m/z* : 302 (M^+, molecular ion), 268, 185, 128, 77.

Results and Discussion

The ethanolic extract of the *n*-hexane soluble fraction of *L. erythrocarpa* stem bark was subjected to Sephadex LH-20 and/or silica gel column chromatography to obtain three chalcones and a stilbene.

The ^{1}H-NMR spectra of compound **1** showed four methoxyl groups at δ 3.82, 3.83 3.85 and δ 4.03 and a set of *trans*-olefinic protons at δ 7.62 and δ 7.65 (each *d*, *J* = 15.5 Hz), which were assigned as H-β, and H-α, re spectively. A total of 19 carbons were appeared in the ^{13}C-NMR spectrum which included four methyl, seven me thine and eight quaternary carbons. The ^{13}C-NMR spe ctrum also showed a carbonyl carbon at δ 195.5 (C=O) (Markham, 1982). These chemical shifts of carbon signals were assigned in comparison with literature values (Leong

Table 1. ^{1}H- and ^{13}C-NMR data of compounds 1 and 2

Position	1[a]		2[a]	
	^{13}C	^{1}H	^{13}C	^{1}H
1	136.4	-	136.4	-
2	129.4	7.64 (2H, dd, *J* = 1.8, 7.6)	129.9	7.70 (2H, dd, *J* = 1.8, 7.6)
3	130.2	7.41 (3H, m)	129.2	7.44 (3H, m)
4	131.7	7.41 (3H, m)	131.1	7.44 (3H, m)
5	130.2	7.41 (3H, m)	129.2	7.44 (3H, m)
6	129.4	7.64 (2H, dd, *J* = 1.8, 7.6)	129.9	7.70 (2H, dd, *J* = 1.8, 7.6)
1'	114.3	-	105.5	-
2'	152.4	-	153.8	-
3'	151.3	-	129.8	-
4'	138.6	-	162.9	-
5'	140.2	-	92.8	6.13 (1H, s)
6'	153.5	-	160.3	-
α	128.3	7.65 (1H, d, *J* = 15.5)	128.2	8.27 (1H, d, *J* = 15.5)
β	145.6	7.62 (1H, d, *J* = 15.5)	143.2	7.82 (1H, d, *J* = 15.5)
C=O	195.5	-	193.5	-
OMe	61.8	3.82 (3H, s)	56.4	3.75 (3H, s)
OMe	61.9	3.83 (3H, s)	61.2	3.92 (3H, s)
OMe	62.0	3.85 (3H, s)	-	-
OMe	62.6	4.03 (3H, s)	-	-

[a]Spectra were measured in CD_3OD at 500 and 125 MHz for ^{1}H- and ^{13}C-, respectively.

Table 2. ^{1}H- and ^{13}C-NMR data of compounds 3 and 4

Position	3[a]		4[a]	
	^{13}C	^{1}H	^{13}C	^{1}H
1	136.9	-	165.3	-
2	127.5	7.56 (2H, d, J = 7.0)	150.5	-
3	128.5	7.34 (2H, m)	187.6	-
4	128.6	7.29 (1H, m)	149.3	-
5	128.5	7.34 (2H, m)	184.9	-
6	127.5	7.56 (2H, d, J = 7.0)	105.5	-
1'	100.4	-	136.7	-
2'	155.5	-	129.9	7.66 (2H, dd, J = 1.5, 8.7)
3'	140.2	-	128.9	7.45 (3H, m)
4'	178.4	-	130.9	7.45 (3H, m)
5'	153.5	-	128.9	7.45 (3H, m)
6'	159.8	-	129.9	7.66 (2H, dd, J = 1.5, 8.7)
α	127.9	8.10(1H, d, J = 15.5)	122.3	8.00 (1H, d, J = 15.8)
β	146.9	7.46 (1H, d, J = 15.5)	141.4	7.58 (1H, d, J = 15.8)
C=O	191.6	-	-	-
OMe	59.2	3.99 (3H, s)	60.2	4.09 (3H, s)
OMe	-	-	60.3	4.16 (3H, s)
OMe	-	-	64.6	4.17 (3H, s)

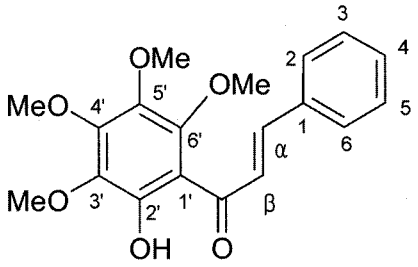
[a]Spectra were measured in CD_3OD at 500 and 125 MHz for ^{1}H- and ^{13}C-, respectively.

et al., 1998). Thus, the chemical structure of the compound 1 was identified as 2'-hydroxy-3',4',5',6'-tetra-methoxychalcone, named as kanakugiol.

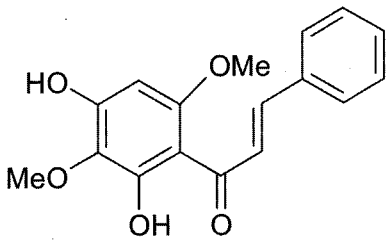
The ^{1}H-NMR signals of the compound 2 for a set of *trans*-olefinic protons at δ 7.82 and δ 8.27 (each d, J = 15.5 Hz) confirmed the existence of the chalcone necleus. A singlet peak appeared at δ 6.13 (1H) which assigned to H-5'. The ^{1}H-NMR spectrum exhibited two methoxy groups at δ 3.75 (3H, s) and δ 3.92 (3H, s), and de termined the position of the methoxy group to be on C-6' and C-3' by using the HMBC spectrum which showed cross peaks between δ 3.75 (3H, s) and C-6' (δ 56.4) and δ 3.92 (3H, s) and C-3' (δ 61.2). The ^{13}C-NMR and HMQC spectra showed the presence of two methoxyls, eight methines and seven quaternary carbons. Using the ^{13}C-NMR and HMQC spectrum, each carbon was ass igned as shown in Table 1 and carboxyl carbon C=O at δ 193.5, respectively. These chemical shifts of carbon signals were assigned in comparison with literature values (Maradufu and Ouma, 1978). Thus, the structure of the compound **2** was identified as 2',4'-dihydroxy-3',6'-di methoxychalcone.

The compound **3** was isolated as a yellow oil. The ^{1}H-NMR spectrum showed signals for five aromatic protons, two olefinic protons and one methoxy methyl proton. A total of 16 carbons appeared in the ^{13}C-NMR spectrum which included one methyl, seven methines and eight quaternary carbons. The ^{13}C-NMR spectrum showed a carbonyl carbon at δ 191.6 (C=O). The corresponding carbons were identified by HMQC as seven methine carbon atoms at δ 127.5 (C-2, 6), δ 127.9 (C-α), δ 128.5 (C-3, 5), δ 128.6 (C-4) and δ 136.9 (C-1), respe ctively. On the basis of data, the structure of the compound **3** was established as 2',4',5',6'-tetrahydroxy-3'-methoxy chalcone.

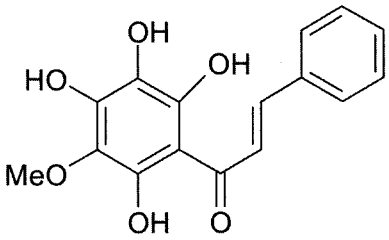
The ^{1}H-NMR spectrum of the compound **4** exhibited the signals of a set of *trans*-coupled olefinic protons at δ 7.58 (^{1}H, *d*, J = 15.8 Hz, H-β) and δ 8.00 (^{1}H, *d*, J = 15.8 Hz, H-α) (Silverstein *et al.*, 1991) and three methoxyl groups at δ 4.09 (3H, s, H-OCH_3), δ 4.16 (3H, s, H-OCH_3) and δ 4.17 (3H, s, H-OCH_3). The correlations of protons to the respective carbons were clarified with the aid of HMQC spectrum. The ^{1}H-^{1}H COSY spectrum revealed the connectivities between C-α/C-β, C-2/C-3 and C-5/ C-6. The ^{13}C-NMR spectrum and DEPT (45°, 90° and 135°) experiments showed the presence of seven quaternary carbons, seven methine and three methyl groups. On the basis of the above findings, the structure of the compound **4** was established as 5,6-dihydroxy-2,3,4-tri methoxystilbene. This is the first report on the isolation



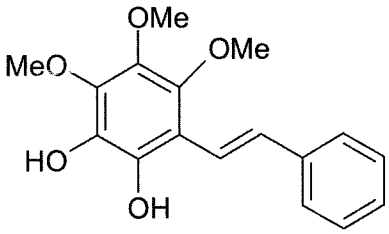
2'-hydroxy-3'4',5',6'-tetramethoxychalcone (1)



2',4'-dihydroxy-3',6'-dimethoxychalcone (2)



2',4',5',6'-tetrahydroxy-3'-methoxychalcone (3)



5,6-dihydroxy-2,3,4-trimethoxystilbene (4)

Fig. 1. The isolated compounds from the stem bark of *L. erythrocarpa*.

of these compounds from the *L. erythrocarpa*.

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