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Flavonoids from *Codonopsis lanceolata* Leaves

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Abstract—For the investigation of resources in *Codonopsis* species, the studies were carried out to evaluate the pharmaco-constituents from the leaves of *Codonopsis lanceolata* (Campanulaceae) whose roots have been used to antitussive, expectorant, detoxicate, tonic, edible, etc. as a folk medicine in Korea.

From the EtOAc and BuOH fractions of MeOH extract, three flavonoid compounds, luteolin-7-O-β-D-glucopyranoside($C_{21}H_{20}O_{11}$, mp 254~255°, compound 1), luteolin-5-O-β-D-glucopyranoside($C_{21}H_{20}O_{11}$, mp 279~281°, compound 2) and luteolin ($C_{15}H_{10}O_6$, mp 327~330°, compound 3) were isolated and identified on the basis of their physico-chemical properties and spectroscopic evidences(UV, IR, ^{1}H-NMR, ^{13}C-NMR, MS etc.) in comparison with authentics respectively.

Keywords—*Codonopsis lanceolata* • Campanulaceae • leaves • luteolin-7-O-β-D-glucopyranoside • luteolin-5-O-β-D-glucopyranoside • luteolin

Codonopsis lanceolata root is natural drug well known for its antitussive, expectorant, detoxicate and tonic effects and also used as a eating agent[1].

Chemical studies of roots on *Codonopsis* species have been focused on lipophilic substances[2,3].

For the investigation of herbal drug from the discarded part of medicinal plant, from aerial part of *Codonopsis lanceolate* we have isolated and identified three known luteolin type flavonoids such as luteolin(3), luteolin-7-O-β-D-glucopyranoside(1) and luteolin-5-O-β-D-glucopyranoside (2) by comparing their spectral data and authentics, respectively.

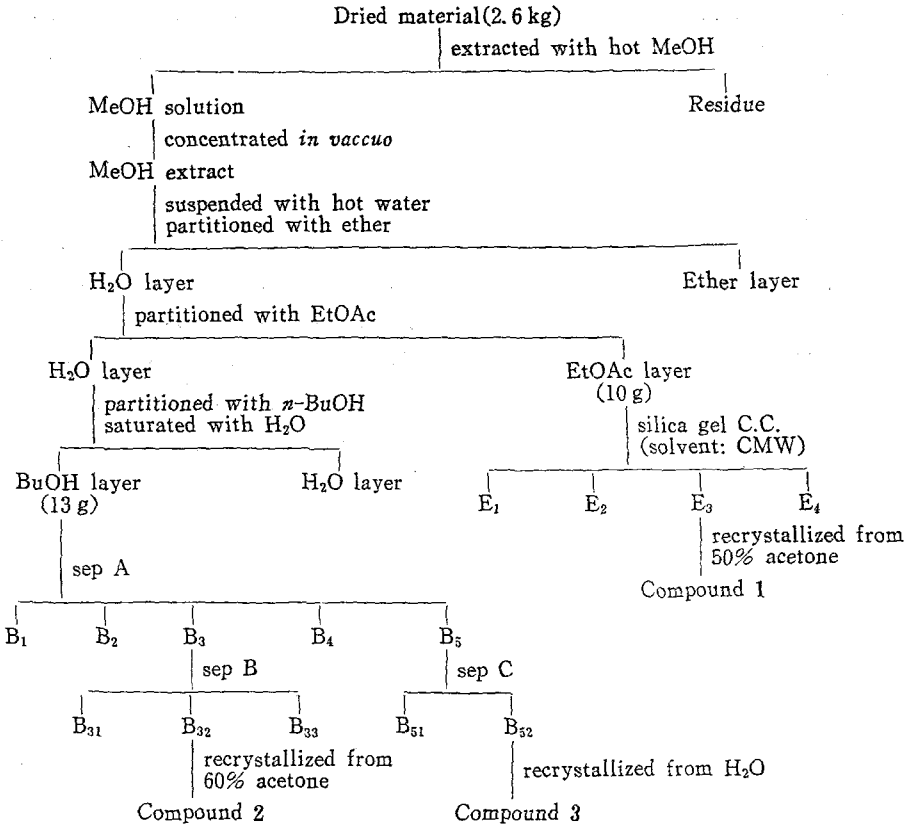
This paper describes possibility of medicinal resouces of aerial part from *Codonopsis lanceolata*.

Materials and Methods

Instruments—Melting point was obtained on Electrothermal IA 8100. IR and UV spectra were measured on a Shimadzu IR-435(Japan) and Varian Cary-3 spectrophotometer(USA) respectively. ^{1}H-NMR(200 MHz) and ^{13}C-NMR (50 MHz) were recorded on a Bruker AM-200 NMR spectrometer(Germany). EI-MS was taken on a GC-MS/MS-DS, TSQ-700 mass spectrometer(France) by the direct inlet method. Acid hydrolysis of glycosides and identification of the resulting monosaccarides by gas-liquid chromatography(GLC) were conducted in the usual manner(Shimadzu GC-14A, Japan).

Plant material—The leaves of *Codonopsis lanceolata* were collected in September(1991) at Keum-kog of Kyung Gi Do. The voucher specimen was deposited at Department of Medicinal Botany, College of Pharmacy, Chung-Ang University.

Extraction and isolation—The dried material (2.6 kg) was extracted with MeOH. The MeOH



Scheme I. Extraction and isolation of compounds 1, 2 and 3
1) CMW; $CHCl_3$-MeOH-H_2O=60 : 35 : 8
2) sep A; Sephadex LH-20(solvent: H_2O→MeOH)
3) sep B; Sephadex LH-20(solvent: EtOH)
4) sep C; Sephadex LH-20(solvent: MeOH)

extract(500 g) *was suspended* in H_2O and *partitioned* with ether, EtOAc and *n*-BuOH, successively and then EtOAc extract was chromatographed over a silica gel column with $CHCl_3$-MeOH-H_2O(in heterogenouse) to afford E_1→E_4. Compound **1** was obtained from E_3 by being recrystallized from 50% acetone. Compound **2** and compound **3** were isolated by Sephadex LH-20 column chromatography with gradient type of MeOH from BuOH extract as Scheme I.

Compound 1—Crystallized from MeOH to yield compound **1** as yellow powder. mp 254~255°; IR, ν_{max}(cm^{-1}) 3450(OH), 1647(C=O), 1608, 1507, 1415(aromatic C=C), 1012(glycosidic C-O); UV, λ_{max}(nm) 254, 347; EI-Mass (*m/z*) 448[M]$^+$, 286[M-Hexose]$^+$, 153, 134; ^{1}H-NMR(200 MHz, DMSO-d_6): 13.0(1H, s, 5-OH), 7.49(1H, dd, *J*=1.7, 8.4Hz, H-6′), 7.45 (1H, d, *J*=1.7Hz, H-2′), 6.90(1H, d, *J*=8.4 Hz, H-5′), 6.73(1H, s, H-3), 6.79(1H, d, *J*=2.1Hz, H-8), 6.46(1H, d, *J*=2.1Hz, H-6), 5.10(1H, d, *J*=7.0Hz, anomeric H); ^{13}C-NMR: see Table I.

Compound 2—Crystallized from MeOH to yield compound **2** as yellow powder. mp 279~281°; $[\alpha]_D^{20}$ −32.0° (c=0.25, pyridine); IR, ν_{max}(cm^{-1}) 3400(OH), 2920(C-H), 1650(C=O), 1600, 1500, 1420(aromatic C=C), 1080 (glycosidic C-O); UV, λ_{max}(nm) 251, 348; EI-Mass(*m/z*) 448[M]$^+$, 286[M-Hexose]$^+$, 153, 134; ^{1}H-NMR(200 MHz, DMSO-d_6): 7.46(1H, dd, *J*=1.7Hz, 8.5Hz, H-6′), 7.39(1H, d, *J*=

Table I. ^{13}C-NMR spectral data of compound 1, 2 and 3

Carbon No.	1[a]	2[b]	3[b]
2	165.3	162.8	164.1
3	104.1	105.9	103.1
4	182.8	177.2	181.8
5	162.6	158.9	161.7
6	100.6	104.7	99.0
7	163.9	161.6	164.3
8	95.3	98.4	94.0
9	157.8	158.5	157.4
10	106.6	108.4	104.0
1′	122.7	121.7	121.7
2′	114.6	113.3	113.5
3′	147.8	145.9	145.9
4′	151.8	149.5	149.9
5′	116.9	116.2	116.2
6′	119.7	118.8	119.2
Glucose			
1″	100.6	104.5	
2″	74.8	73.6	
3″	78.4	77.8	
4″	71.2	69.9	
5″	79.2	75.8	
6″	62.4	61.1	

a) solvent: pyridine-d_5(50 MHz)
b) solvent: DMSO-d_6(50 MHz)

2.1Hz, H-8), 6.46(1H, d, J=2.1Hz, H-6), 5.10(1H, d, J=7.0Hz, anomeric H); ^{13}C-NMR: see Table I.

Hydrolysis of compound 1 and compound 2—Compounds 1 and 2 (each 20 mg) were hydrolyzed, respectively by using the method in general procedure. The hydrolysate was diluted with H_2O and extracted with $CHCl_3$. The residue from the $CHCl_3$ extract was chromatographed on the Sephadex LH-20 column using an eluting solvent system of EtOH. The elutes were concentrated respectively, and the residues were recrystallized from MeOH to give the same aglycone of compound 3, yellow needle, mp 327~330°; IR, ν_{max}(cm^{-1}) 3380(OH), 1655(C=O), 1615, 1580, 1437(aromatic C=C); EI-Mass(m/z) 286 [M]$^+$, 153, 134; ^{1}H-NMR(200MHz, DMSO-d_6): 7.50(1H, dd, J=1.7, 8.5Hz, H-6′), 7.43 (1H, d, J=1.7Hz, H-2′), 6.95(1H, d, J=8.5 Hz, H-5′), 6.73(1H, s, H-3), 6.79(1H, d, J=2.1Hz, H-8), 6.45(1H, d, J=2.1Hz, H-6); ^{13}C-NMR: see Table I, which was identified as luteolin by direct comparison with an authentic sample. After being neutralized with Amberlite MB-3, the filtrate was concentrated to a small volume and examined by TLC and GC, to show the presence of glucose(t_R 15.8) from compounds 1 and 2.

Compound 3—Crystallized from MeOH to yield compound 3 as yellow needle crystal. mp 327~330°; $[\alpha]_D^{20}$ −44.0° (c=0.25 pyridine); IR, ν_{max}(cm^{-1}) 3379(OH), 1653(C=O), 1615, 1578, 1437(aromatic C=C); UV, λ_{max}(nm) 255, 350; EI-Mass(m/z) 286[M]$^+$, 153, 134; ^{1}H-NMR(200 MHz, DMSO-d_6): 12.95(1H, s, 5-OH), 7.50(1H, dd, J=1.7, 8.5Hz, H-6′), 7.40(1H, d, J=1.7Hz, H-2′), 6.90(1H, d, J=8.5Hz, H-5′), 6.67(1H, s, H-3), 6.45(1H, d, J=1.7Hz, H-8), 6.19(1H, d, J=1.7Hz, H-6); ^{13}C-NMR: see Table I.

Results and Discussion

From the EtOAc and n-butanol of the methanol extract of the leaves, three compounds of flavonoid were isolated by silica gel C.C. and Sephadex LH-20 C.C.

Two compounds(compound 1, 2) were O-glycosides of luteolin type and the other compound (compound 3) was luteolin.

Compound 1—Yellow powder, mp 254~255°C, was insoluble in $CHCl_3$, ether and acetone but soluble in MeOH. Compound 1 was detected by $FeCl_3$ and Mg-HCl as a positive reaction, suggesting it to be a flavonoid. The bands of broad hydroxyl absorption at 3345 cm^{-1} and conjugated carbonyl absorption of γ-pyrone at

1647 cm^{-1} and strong absorptions of aromatic ring at 1608, 1507 and 1415 cm^{-1} in IR spectrum. UV λ_{max} of compound **1**, 254 and 347 nm (in MeOH), were appeared in a typical pattern of flavone, and also measuring to add various shift reagents, it was showed typical pattern of luteolin type[4~6]. The EI-MS spectrum showed a molecular ion at *m/z* 448 and other fragment ions at *m/z* 286 [M-hexose]$^+$, 153 [RDA, A ring]$^+$ and 134 [RDA, B ring]$^+$ [7]. The ^{1}H-NMR spectrum (DMSO-d_6) showed a double doublet signal at δ7.49(1H, J=1.7, 8.4Hz, H-6′), a doublet signal at δ7.45(1H, J=1.7Hz, H-2′) and a doublet signal at δ6.90(1H, J=8.4Hz, H-5′) on B ring. The *meta* coupled doublet signals at δ 6.46 and 6.79 were assignable to H-6(J=2.1Hz) and H-8(J=2.1Hz) and the signal at δ6.73 to H-3. And anomeric proton signal of sugar showed β conjugation at δ 5.10(J=7.0Hz). ^{13}C-NMR spectrum(pyridine-d_5) showed carbonyl signal at δ 182.8(C-4) in Table I. And also C-3′ and C-4′ of B ring revealed at δ 147.8 and δ 151.8 respectively[8]. Carbon signals of sugar were confirmed at δ 100.6, 74.8, 78.4, 71.2, 79.2 and 62.4[9]. On the hydrolysis of acid, compound **1** was confirmed to luteolin and glucose by TLC and GC.

These data suggested that compound **1** was luteolin-7-O-β-D-glucopyranoside and melting point and other physical data were identical with those of an authentic sample.

Compound 2—Yellow powder, mp 279~281 °C, was insoluble in $CHCl_3$ and ether but soluble in MeOH. Compound **2** was detected by $FeCl_3$ and Mg-HCl as a positive reaction, suggesting it to be a flavonoid. The pattern of IR and UV spectra was similar to that of compound **1**. Therefore the structure of compound **2** indicated same luteolin type as compound **1**. And the EI-MS spectrum was also similar to that of compound **1**. The ^{1}H-NMR(DMSO-d_6) and ^{13}C-NMR(DMSO-d_6) spectra were similar to those of compound **1**, but hydroxyl signal(5-OH) at 10~13 ppm was not appeared in ^{1}H-NMR, and carbon signals at A and C rings were shifted by influence of glycosidation of sugar as that signal of C-5 was upfield shift(2.8 ppm) and adjacent carbon(C-4) revealed downfield shift (4.6 ppm) in the ^{13}C-NMR.

These data suggested that compound **2** was luteolin-5-O-β-D-glucopyranoside and melting point and other physical data were identical with those of an authentic sample.

Compound 3—Yellow needle crystal, mp 327 ~330 °C, was soluble in $CHCl_3$, acetone and MeOH. Compound **3** was detected by $FeCl_3$ and Mg-HCl as a positive reaction, suggesting it to be a flavonoid. The pattern of IR and UV spectra was similar to those of compound **1**. The EI-MS spectrum showed a molecular ion at *m/z* 286(M^+) and other fragment ions at *m/z* 153 and 134. The ^{1}H-NMR(DMSO-d_6) and ^{13}C-NMR(DMSO-d_6) spectra were also similar to those of compound **1**, but no sugar signals appeared.

These data suggested that compound **3** was luteolin and melting point and other physical data were identical with those of an authentic sample.

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