목재공학 23(2): 94~99(原著)
Mokchae Konghak. 23(2): 94~99(Original). 1995

Flavonoid Extractives of *Populus albaglandulosa*[*1]

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현사시나무의 후라보노이드 抽出成分[*1]

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ABSTRACT

현사시나무의 목질부와 수피부를 아세톤-물(1:1)의 혼합용액으로 추출하고 Sephadex LH-20로 충진한 칼럼을 사용하여 4개의 후라보노이드, 즉 (+)-catechin, (+)-dihydroquercetin, eriodictyol 및 (+)-dihydrokaempferol을 단리하고 ^{13}C-NMR과 ^{1}H-NMR 스펙트럼을 이용하여 그 구조를 규명하였다. 단리된 화합물의 A-환은 모두 후로로그루시놀형으로, B-환은 카테콜형 또는 페놀형으로 구성되어 있으며 (+)-dihydroquercetin, (+)-dihydrokaempferol 과 eriodictyol은 포플라속의 수종에서는 처음으로 단리되었다.

Keywords : *Populus albaglandulosa,* wood, bark, fravonoid extractives, ethylacetate soluble

1. INTRODUCTION

Populus albaglandulosa, which has been established by hybridization between *P. alba* L. and *P. glandulosa* Uyeki in Korea, is a tree species growing well at any place where soil moisture content and soil oxygen concentration are proper. The wood consists of dark brown heartwood and pale yellow sapwood with indistinct annual ring. Its average specific gravity is less than 0.5 and the mechanical strength is also comparatively low[16,17]. The tree is reported to have high decay and insect resistance and sometimes is used to pulp wood. Its wood and bark are also known to contain a large amount of salicylic acids and their derivatives in addition to small amount of phenolic compounds. However, *Populus* extractive has never been investigated in domestic and its chemistry also is not still fully understood. This study was carried out to isolate flavonoid compounds of the species, one of the main domestic hardwoods, and to obtain useful basic information for chemical utilization.

2. EXPERIMENTAL METHOD

2.1 Equipments

^{13}C-NMR and ^{1}H-NMR spectra were obtained from a Varian Gemini 200 NMR spectrometer with samples dissolved in MeOH-d_4 and Acetone-d_6 and chemical shifts are given in values. Chromatographic columns were packed with

*1 접수 1995년 2월 20일 Received February 20, 1995
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Sephadex LH-20 and eluents were collected using a Gilson FC 204 fraction collector.

Analytical thin layer chromatography (TLC) was performed on cellulose plates (Merk DC-plastikfolien cellulose) and developed with t-BuOH-HOAc-H_2O (3:1:1, solvent A) HO Ac-H_2O(3:47, solvent B). Visualization was done by illuminating ultraviolet light or by spraying vanillin-HCl-EtOH(60:0.15:6) followed by heating.

2.2 Extraction and isolation

Air dried fresh wood and bark of a 15-year-old *Populus albaglandulosa* collected in February 1992 in the campus forest of Kangwon National University were prepared to fine particles by grinding mill. Wood(2kg) and bark(3kg) particles were extracted three times with acetone-water(1:1, v/v) by soaking at room temperature for 72hr each time in 20 *l* glass jar.

The extract was combined, and concentrated on a rotary evaporator under reduced pressure. The concentrated extract was separated into two fractions, ethylacetate and water soluble, using ethylacetate and water on a separatory funnel.

Each fraction was concentrated, freezed and then freeze dried to give tan-colored powder. Ethylacetate and water soluble freeze-dried powder were 134.4g and 246.6g from bark particles, respectively, and 37.8g and 41.5g from wood, respectively.

About 10g of each fraction was applied to a column(3×100cm) and the procedure was repeated 2~3 times to get enough starting materials for further steps. Then the column was washed with ethanol until eluents are almost colorless.

Bark ethylacetate soluble was separated into 5 fractions, B Ⅰ(33.3g), B Ⅱ(2.8g), B Ⅲ(1.2g), B Ⅳ(0.7g) and B Ⅴ(0.2g).

Wood ethylacetate portion was also separated into 5 fractions, W Ⅰ(3.8g), W Ⅱ(7.1g), W Ⅲ(4.2g), W Ⅳ(4.2g) and W Ⅴ(0.2g).

B Ⅲ was rechromatographed to give 4 fractions, B Ⅲ-1(0.25g), B Ⅲ-2(0.63g), B Ⅲ-3(0.1g), B Ⅲ-4(0.01) using EtOH-H_2O(4:1, v/v) as an eluent.

2.2.1 (+)-Catechin, 3',4',5,7-tetrahydroxy flavan-3-ol

B Ⅲ-2 was retreated on a column using aqueous ethanol(2:1, 1:1, 2:3) to give a pure compound, (+)-catechin(30mg). R_f 0.56(solvent A) and 0.42(solvent B).

^{13}C-NMR(ppm, Acetone-d_6): 28.5(C-4), 68(C-3), 82.3(C-2), 95.2(C-8), 96.1(C-6), 100.5(C-10), 115.4(C-2'), 115.9(C-5'), 119.9(C-6'), 131.7(C-1'), 145.6(C-3'), 145.7(C-4'), 156.6(C-7), 157.1(C-5), 157.5(C-9).

^{1}H-NMR (δ, MeOH-d_4) : 2.5 (dd, J=8.2Hz, 16.1Hz, 1H), 2.85(dd, J=5.4Hz, 16.2Hz, 1H), 3.95(m, 1H), 4.55(d, J=7. 6Hz, 1H), 5.8~5.9(dd, J=2.3Hz, 2H), 6.8(m, 3H).

2.2.2 (+)-Dihydroquercetin(taxifolin), 3', 4',5,7-tetrahydroxyflavanonol

B Ⅲ-2 also gave a yellowish material (118mg) by column chromatography using an EtOH-H_2O (3:7, v/v) eluent after washing with various aqueous ethanol. R_f 0.76(solvent A) and 0.38 (solvent B).

^{13}C-NMR(ppm, MeOH-d_4): 73.6(C-3), 85(C-2), 96.4(C-8), 97.4(C-6), 101.9(C-10), 116(C-2'), 116.2(C-5'), 121(C-6'), 130(C-1'), 146.4(C-3'), 147.2(C-4'), 164.6(C-9), 165.4(C-5), 168.8(C-7), 198.5(C-4).

2.2.3 Eriodictyol, 5,7,3',4'-tetrahydroxy flavanone

Eriodictyol was also obtained from B Ⅲ-2 using aqueous ethanol. R_f 0.03(solvent A), 0.88 (solvent B).

^{13}C-NMR(ppm, MeOH-d_4): 44.3(C-3), 80.8(C-2), 96.6(C-8), 97.4(C-6), 103.7(C-10), 115.1(C-2'), 116.7(C-5'), 119.7(C-6'), 129.5(C-1'), 146.9(C-3'), 147.3(C-4'), 165.3(C-9), 165.9(C-5), 168.8(C-7), 198.3(C-4).

2.2.4 (+)-Dihydrokaempferol(aromadendrin), 4',5,7-trihydroxyflavanonol

W Ⅳ was rechromatographed on a column

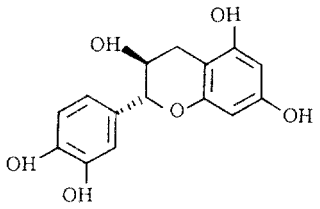
using aqueous methanol and aqueous ethanol to give a purified yellowish compound, aromadendrin(34mg). R_f 0.83(solvent A), 0.32(solvent B).

^{13}C-NMR(ppm, MeOH-d_4): 73.6(C-3), 84.8(C-2), 98.5(C-8), 99.5(C-6), 101(C-10), 116.7(C-3', C-5'), 130(C-1'), 130.8(C-2', 6'), 159(C-4'), 164.7(C-9), 165.5(C-5), 169(C-7), 196.9(C-4).

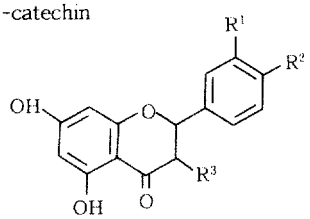
^{1}H-NMR(δ, Acetone-d_6): 4.6(d, J=11.1Hz, 1H), 5.1(d, J=11.2Hz, 1H) 5.95(s, 1H), 5.98(s, 1H), 6.9(d, J=8.1Hz, 2H), 7.42(d, J=8.3Hz, 2H).

3. RESULTS & DISCUSSION

In this study, four flavonoids, (+)-catechin, (+)-dihydroquercetin, (+)-dihydrokaempferol and eriodictyol, were isolated by repeating column chromatography packed with Sephadex LH-20 after extraction with acetone-water(1:1, v/v). Eluents were ethanol, aqueous ethanol and aqueous methanol.



I : (+)-catechin



Ⅱ : R_1=R_2=R_3=OH, (+)-dihydroquercetin
Ⅲ : R_1=R_2=OH, R_3=H, eriodictyol
Ⅳ : R_1=H, R_2=R_3=OH, (+)-dihydrokaempferol

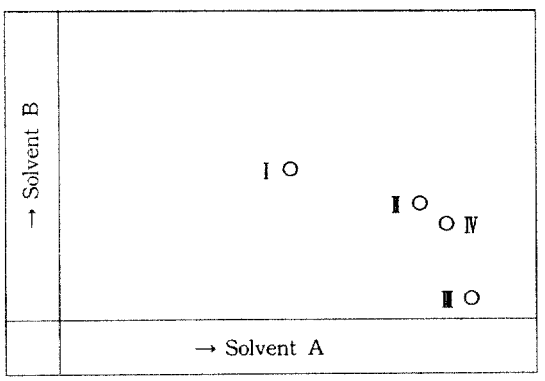


Fig. 1. Two dimensional thin layer chromatogram of isolated compounds.

Characterization of isolated compounds was carried out by one and two dimensional thin layer chromatography(Fig. 1.) and the plates were visualized by spraying vanillin-HCl-EtOH solution after development. ^{13}C-NMR and ^{1}H-NMR spectrum were also very useful tools to determine the structures of the compounds.

3.1 (+)-Catechin, 3',4',5,7-tetrahydroxyflavan-3-ol

Compound I gave a pink to red spot on the plate when visualized with the spraying reagent[13]. R_f values, 0.56(solvent A) and 0.42(solvent B), were very similar to authentic compound characterized by several researchers.

Its ^{13}C-NMR spectrum also showed very similar carbon signals to (+)-catechin with hydroxylation pattern of phloroglucinol A-ring, catechol B-ring and etherocyclic C-ring.

In A-ring, the signals of C-6 and C-8 were 96.2 ppm and 95.1ppm, respectively and C-5, C-7 and C-9 bearing oxygen appeared at 156.5~158ppm. These are very close to phloroglucinol A-ring signals. B-ring signals also showed typical catechol ring resonances. C-1', quaternary carbon, resonated at 131.7ppm and C-2', C-5' and C-6' absorbed at 115.4ppm, 115.9 and 119.9ppm, respectively. The chemical shifts of C-3' and C-4', substituted with hydroxyl group, were 145.6ppm and 145.7 ppm.

In etherocyclic C-ring, C-2, C-3 and C-4 resonances absorbed at 68ppm, 82.3ppm, 28.5ppm, respectively. These peak patterns mean C-4 does not have a carbonyl oxygen and aliphatic methylene C-3 has a hydroxyl substituent.

In ^{1}H-NMR spectrum, multiplet appeared at δ 3.95 were assigned to H-3 splitted by adjacent protons, H-2, axial H-4 and equatorial H-4. Double doublets at δ 2.5 and δ 2.85 corresponded to

axial H-4 and equatorial H-4.

Consequently, this compound was identified to (+)-catechin, 3',4',5,7-tetrahydroxyflavan-3-ol reported by many scientists[3,5,6,7,8,15].

3.2 (+)-Dihydroquercetin(taxifolin), 3',4', 5,7-tetrahydroxyflavanonol

Yellowish compound Ⅱ gave a light pink color on a cellulose plate when sprayed with the detecting solution. R_f values were 0.76(solvent A), 0.38(solvent B) and very similar to standard (+)-dihydroquercetin[11].

^{13}C-NMR spectrum also showed characteristic (+)-dihydroquercetin carbon resonances with hydroxylation pattern of phloroglucinol A-ring and catechol B-ring.

In A-ring, each carbon resonance exhibits very similar signals to typical phloroglucinol A-ring. The hydroxylated carbons, C-5 and C-7, gave signals at 165.4ppm and 168.8ppm and C-9 bearing oxygen at 164.6ppm. The chemical shifts of C-6 and C-8 absorbed at 97.4ppm and 96.4ppm, respectively.

Three resonances of heterocyclic C-ring absorbed at 85ppm, 73.6ppm and 198ppm corresponding to the C-2, C-3 and C-4, respectively.

The peaks of B-ring also showed typical catechol shifts. C-1' absorbed at 130ppm and C-2' and C-5' resonated at 116ppm and 116.2ppm. The hydroxylated C-3' and C-4' resonated at 146.4ppm and 147.2ppm.

In general, C-4 containing carbonyl resonates at 196~198ppm in 5-hydroxylated flavanones but at 190~192ppm in 5-unsubstituted[4].

Therefore, this compound was determined to (+)-dihydroquercetin, 3',4',5,7-tetrahydroxyflavanonol[2,9,12,14]. However, this compound has never been reported in *Populus* although it is a well-known and one of major softwood extractives[10].

3.3 Eriodictyol, 3',4',5,7-tetrahydroxyflavanone

Compound Ⅲ was visualized to a brown spot on a plate after spraying the reagent. R_f values were 0.88(solvent A) and 0.03(solvent B).

^{13}C-NMR spectrum was identical to phloroglucinol A-ring, catechol B-ring, and heterocyclic C-ring bearing oxymethine C-2, aliphatic methylene C-3 and carbonyl C-4.

In A-ring, the chemical shifts of C-6 and C-8 absorbed at 97.4ppm and 96. 6ppm and C-5, C-7 and C-9 bearing oxygen appeared at 165. 9ppm, 168.8ppm and 165.3ppm, respectively. These resonances were the same as typical phloroglucinol A-ring signals of a flavanone.

B-ring signals were proved to typical catechol resonances. C-1' absorbed at 129.5ppm and C-2', C-5' and C-6' at 115.1ppm, 116.7ppm and 119.7ppm and hydroxylated C-3' and C-4' resonated at 146.9ppm and 147.3ppm, respectively.

In C-ring, the resonances of C-2, C-3 and C-4 absorbed at 80.8ppm, 44.3ppm and 198.3ppm, respectively. 44.3ppm of C-3 means it is a unsubstituted methylene carbon.

However, most of the ^{13}C-NMR signals were downfield shifted by 2~3ppm compared to reference data[1] using dimethylsulfoxide as a solvent and it seems to be due to the solvent effect.

Finally, this compound was characterized to eriodictyol, 3',4',5,7-tetrahydroxyflavanone[1,9] and also firstly characterized in *Populus*[10].

3.4 (+)-Dihydrokaempferol(aromadendrin), 4',5,7-trihydroxyflavanonol

Yellow compound Ⅳ isolated from wood fraction Ⅳ gave R_f values, 0.83(solvent A) and 0. 32(solvent B)[11].

^{13}C-NMR showed typical signals of phloroglucinol A-ring, phenolic B-ring and heterocyclic C-ring. A-ring shifts showed very similar carbon peaks to phloroglucinol of compound Ⅱ and Ⅲ mentioned above. C-6 and C-8 appeared at 99.5ppm and 98.5ppm and C-5, C-7 and C-9 at 165.5ppm, 169ppm and 164.7ppm, respectively.

B-ring gave only four signals, 116.7ppm, 130ppm, 130.8ppm and 159ppm. These resonances mean the aromatic B-ring must have two pairs of symmetric structures. C-2' and C-6' absorbed at 130.8ppm, C-3' and C-5' at 116.

7ppm and hydroxyl containing C-4' at 159ppm. C-1', a quaternary carbon, gave a signal at 130ppm.

Furthermore, ^{1}H-NMR spectrum also gave some more detail information on the symmetric structure of B-ring. Two doublet signals at δ 6.9 and δ 7.42 were corresponded to C-2'(C-6') and C-3'(C-5') , respectively. Their coupling constants were 8.1Hz and 8.3Hz and mean ortho coupled C-2' and C-3' or C-5' and C-6'. Structure determination using ^{1}H-NMR and ^{13}C-NMR spectra were also similar to the feature of phenolic B-ring.

In heterocyclic C-ring, C-2, C-3 and C-4 appeared at 84.8ppm, 73.6ppm and 196.9ppm, respectively. These signals were very much similar to (+)-dihydroquercetin mentioned above. In ^{1}H-NMR spectrum, H-2 and H-3 appeared at δ 5.1(d, J=11.2Hz) and δ 4.6(d, J=11.1Hz), respectively.

Finally, this compound was corresponded to (+)-dihydrokaempferol, 4',5,7-trihydroxyflavanonol[11] and also firstly isolated in *Populus*[10].

4. CONCLUSIONS

Populus albaglandulosa wood and bark were extracted with acetone-H_2O(1:1, v/v), then the extract was isolated by repeating column chromatography packed with Sephadex LH-20 and the washing eluents were ethanol, methanol and their aqueous solution.

Three flavonoids, (+)-catechin, (+)-dihydroquercetin and eriodictyol, were from bark fraction and (+)-dihydrokaempferol from wood extractive. Their structures were characterized by thin layer chromatography and NMR spectroscopy. Isolated compounds were composed of phloroglucinolic A-ring and catechol B-ring or phenolic B-ring.

(+)-Dihydroquercetin, eriodictyol and (+)-dihydrokaempferol were firstly isolated from *Populus* genus.

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