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Structural Identification of *Robinia pseudacacia* L. Flavonoids for Wood Adhesive Formulation*1

Young-Soo Bae*2

木材 接着劑 製造를 위한 아까시나무 타닌의 構造糾明*1

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요 약

아까시나무로부터 두종류의 flavanone인 7, 3′, 4′ − trihydroxy flavanone과 3, 7, 3′, 4′, 5′− pentahydroxy flavanonol이 단리되어 ^{13}C NMR에 의해 구조가 규명되었다. flavanone은 resorcinol A-ring과 Catechol B-ring으로, flavanonol은 resorcinol A-ring 및 pyrogallol B-ring으로 구성되고 있다.

1. INTRODUCTION

Robinia pseudacacia L., known as locust tree, is one of abundant forest resources native a large area of Korea. The wood consists of yellow-brown heartwood with a narrow margin of white sapwood[15] and is primarily used in furniture making.[4] This tree has already been the subject of a number of chemical investigations and its flavonoids shown to mostly consist of the resorcinol A-ring and the catechol or the pyrogallol B-ring hydroxylation pattern. Also its heartwood has exceptionally high decay resistance and durability, attributable to the high concentration of flavonoids such as robinetinidol and especially dihydrorobinetin.[15, 16]

However, the chemical constituents of *Robinia pseudacacia* have never been investigated in Korea and its economic potential in wood industry is also underestimated.

This preliminary report describes an examination of the wood of *Robinia pseudacacia* which has resulted in the isolation of its flavonoid constituents and an investigation of the potential for wood adhesive formulation using its chemical contents.

2. EXPERIMENTAL

2.1 General

^{13}C NMR spectra at 20MHz were obtained

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*2. 강원대학교 임과대학, College of Forestry, Kangweon National University, Chuncheon 200−701, Korea

from a Varian FT-80 NMR spectrometer with samples dissolved in MeOH-d_4 solvent and chemical shifts are given in δ-values.

Chromatographic columns were packed with Sephadex LH-20(Pharmacia) and eluents were collected using a linear fraction collector(Buchler LC 200).

Analytical TLC was performed on cellulose plates(Baker-flex cellulose F, J.T. Baker Inc.) and developed with t-BuOH-HOAc-H_2O(3 : 1 : 1, solvent A) and HOAc-H_2O(3 : 47, solvent B). Detection was done by spraying vanillin-HCl-EtOH(60 : 0.15 : 6) followed by heating.

2.2 Extraction and isolation

Air dried fresh wood(2.1kg) of a 18-year-old *Robinia pseudacacia* L., collected in April 1990 in the campus forest of Kangweon National University, was extracted three times with MeOH by soaking the chips at room temperature for a minimum of 72hr each time. The combined extract was concentrated on a rotary evaporator under reduced pressure and freeze dried to give a dark brown solid(42g). This solid was dissolved in H_2O, and the resulting aqueous extract was exhaustively washed with EtOAc. The combined EtOAc extract was washed again with hexane to remove waxy materials(4.9g), evaporated and freeze dried to give a fluffy brown powder(10.3g). The H_2O soluble fraction was filtered to remove precipitate and freeze dried to yield a brown powder(5.2g).

Whole amount of EtOAc soluble materials (10.3g) was applied to a column(3×45cm), and the column was washed with EtOH to yield fraction Ⅰ(0.9g), fraction Ⅱ(3.2g), fraction Ⅲ(1.8g) and fraction Ⅳ(3.0g) until the eluent was almost colourless.

2.2.1 (−)-butin, 7, 3′, 4′-trihydroxy flavanone

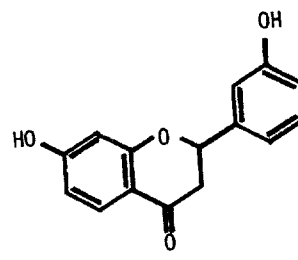
Fraction I was retreated on a column using MeOH-H_2O(1 : 1) to give a chromatographically homogeneous product, 7, 3′, 4′-trihydroxy flavanone(0.14g). R_f 0.75(solvent A) and 0.20 (solvent B). ^{13}C NMR(ppm, MeOH-d_4) : 45(C-3), 81(C-2), 104(C-8), 112(C-6), 115(C-2′)′, 115.4(C-10), 116.8 (C-5′), 119.6(C-6′), 130(C-1′), 132.4 (C-5), 146.2(C-3′), 146.8(C-4′), 166 (C-9), 167(C-7), 194(C-4).

2.2.2 (+)-dihydrorobinetin, 3, 7, 3′, 4′, 5′-pentahydroxy flavanonol

Fraction Ⅱ was worked-up by repeated column chromatography, first using MeOH-H_2O(1 : 1) and then MeOH-H_2O(1 : 3) to yield purified single compound, 3, 7, 3′, 4′, 5′-pentahydroxy flavanonol(2.6g). R_f 0.60(solvent A) and 0.40(solvent B). ^{13}C NMR(ppm, MeOH-d_4) : 74(C-3), 85(C-2), 103.8 (C-8), 108(C-2′ and C-6′), 112(C-6), 1(C-10), 129.2(C-5), 130(C-1′), 134.7 (C-4′), 146.5(C-3′ and C-5′), 164.8(C-9), 166.2(C-7), 194.2(C-4).

3. RESULTS AND DISCUSSION

In this preliminary work, two flavanoids, (−)-butin and (+)-dihydrorobinetin, were obtained by repeated column chromatography with Sephadex LH-20 of the EtOAc soluble portion of the wood extractive using EtOH and MeOH-H_2O(1 : 1 and 1 : 3) as eluents.



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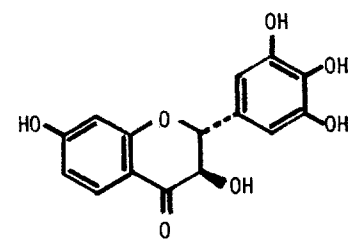

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3.1 (−)-Butin, 7, 3′, 4′-trihydroxy flavanone

Compound 1 exhibited an orange to red colour on TLC when sprayed with vanillin −HCl−EtOH solution. R_f values(Figure 1), 0.75(solvent A) and 0.20(solvent B), were identical to a sample isolated by Roux and Paulus.[14, 15]

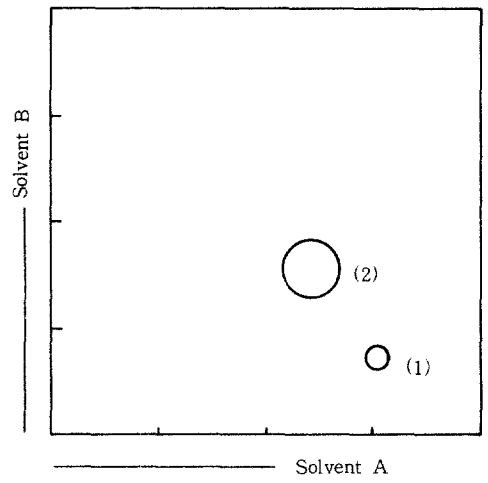

Fig. 1. Two dimensional chromatogram of compound 1 and 2.

Its ^{13}C NMR spectrum(Figure 2) showed very similar carbon signals to(−)-butin, 7, 3′, 4′-trihydroxy flavanone, with the hydorxylation pattern of the resorcinol A-ring and the catechol B-ring.

The heterocyclic C-ring is composed of three resonances, and oxymethine(C−2), an aliphatic methylene(C−3), and a carbonyl (C−4) and their resonances absorbed at 45, 81 and 194ppm corresponding to the C−3, C−2 and C−4 respectively. In general, the chemical shift of the C−3 resonance does not show any dependence upon the substitution in aryl A-and B-ring of flavanones and the C-2 exerts dependence particulary upon the substitution at C−2′ and C−6′ position of arvl B-ring as it resonates at 80±1ppm in 2′−and

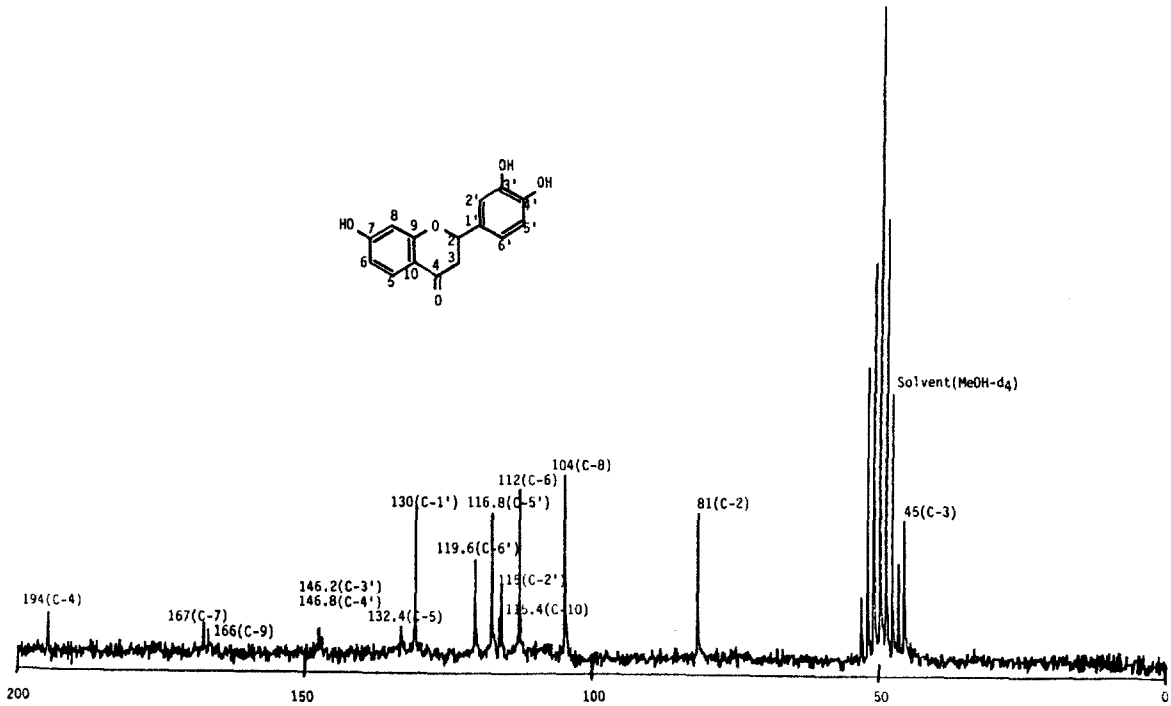

Fig. 2. ^{13}C NMR spectrum of compound 1.

6′−unsubstituted flavanones whereas at 76±1ppm in 2′−or 6′−oxy substituted flavanones.[1, 12] The carbonyl resonance(C−4) exhibits dependence upon the absence or the presence of substituent at C−5 position. In case of 5-unsubstituted ones, the C−4 resonates at 190−192ppm whereas at 196−198ppm in 5-hydroxylated flavanones.[3] Therefore, these heterocyclic ring signals were very close to the chemical shifts of C-ring carbons of flavanones.

In aryl B-ring, the carbon signals showed typical catechol ring resonances. The aryl B-ring signals of flavanones are not affected by the substituents in aryl A-ring[1] and the C−1′, a quaternary carbon, resonated at 103ppm and the resonances of the remaining methine carbons appeared at 115, 116.8 and 119ppm corresponding to the C−2′, C−5′ and C−6′ respectively. The chemical shifts of the C−3′ and C−4′ bearing hydroxy substituents resonated at 146.2 and 146.8ppm.

The chemical shifts of the C−6 and C−8 in aryl A-ring are 96.1 and 95.1ppm in 5, 7-dihydroxy flavanones.[1] In this compound, the C−6 and C−8 resonances absorbed at 112 and 104ppm and these chemical shifts mean this aryl A-ring has one hydroxy substituent at the C−7 position. The oxy-substituted carbons showed two lowfield signals at 166 and 167ppm corresponding to the C−9 and C−7 respectively. Finally, the C−5 appeared at 132.4ppm and the C−10, a quaternary carbon, gave a signal at 115.4ppm.

Consequently, this compound was identified as (−)-butin, 7, 3′, 4′-trihydroxy flavanone, which the structure is the same as the reports of many researchers.[2, 5, 7, 8, 9, 10, 13, 17]

3.2 (+)-Dihydrorobinetin, 3, 7, 3′, 4′, 5′-pentahydroxy flavanonol

Compound 2 gave a yellow colour on TLC when sprayed with the detecting reagent. R_f (Figure 1) was 0.60(solvent A) and 0.40(solvent B) and these values were very similar to a sample isolated by Roux and Paulus.[15]

^{13}C NMR spectrum(Figure 3) showed characteristic carbon resonances of (+)-dihydrorobinetin with the hydroxylation pattern of the resorcinol A-ring and the pyrogallol B-ring.

Three resonances of the heterocyclic C-ring absorbed at 85, 74 and 194.2ppm corresponding to the C−2, C−3 and C−4 respectively. Flavanonols are 3-hydroxy substituted flavanones and hydroxyl substitution at C−3 position of flavanones leads to about 30 and 5ppm downfield shifts of the C−3 and C−2 resonances in respective whereas C−4 resonance remain almost uninfluenced.[1] Therefore, the C−2 was represented by a downfield signal absorbed at 85ppm whereas the C−3 appeared at 74ppm. Also the chemical shift of the C−2 exerts dependence upon the substitution of C−2′ or C−6′ position of aryl B-ring by an oxy substituent. In 2′-unsubstituted flavanonols, C−2 resonates at 82−86 ppm whereas at 76−79ppm in 2′-oxy substituted one.[1, 12] Thus, the chemical shift of the C−2, 85ppm, means the absence of the 2′- or 6′-oxy substituent in aryl B-ring of this compound.

In aryl B-ring, the C−1′ resonance absorbed at 130ppm and as mentioned above, the C−2′ and C−6′ are unsubstituted aryl methine carbons and gave one strong signal at 108ppm. Also the C−3′ and C−5′ resonances appeared at 146.5ppm as singlet and the chemical shift of the C−4′ resonance showed at 134.7ppm.

Therefore, the aryl B-ring of this compound must be 3′, 4′, 5′-trihydroxy phenyl because the ring with a pyrogallol subsstitution pattern is symmetrical and consists of 4 signals whereas 6 signals in the catechol ring.

The aryl A-ring gave very similar signals to the resorcinol A-ring of (−)-butin. The 7-hydorxy substituted carbon gave a signal at 166.2ppm and the C−9, an oxygen bearing

quaternary carbon, appeared at 164.8ppm. The chemical shift of the C−5 resonance absorbed at 129.2ppm and the C−10, another quaternary carbon, showed a signal at 113.1ppm. The resonances of the C−6 and C−8 absorbed at 112 and 103ppm respectively and these resonance values can be used for predicting the presence of the 7-hydroxy substituent.

As a result of the above data, this compound was identified as (+)-dihydrorobinetin, 3, 7, 3′, 4′, 5′-pentahydroxy flavanonol, which has the same structure as the compound reported by several researchers.[6, 8, 9, 11, 13]

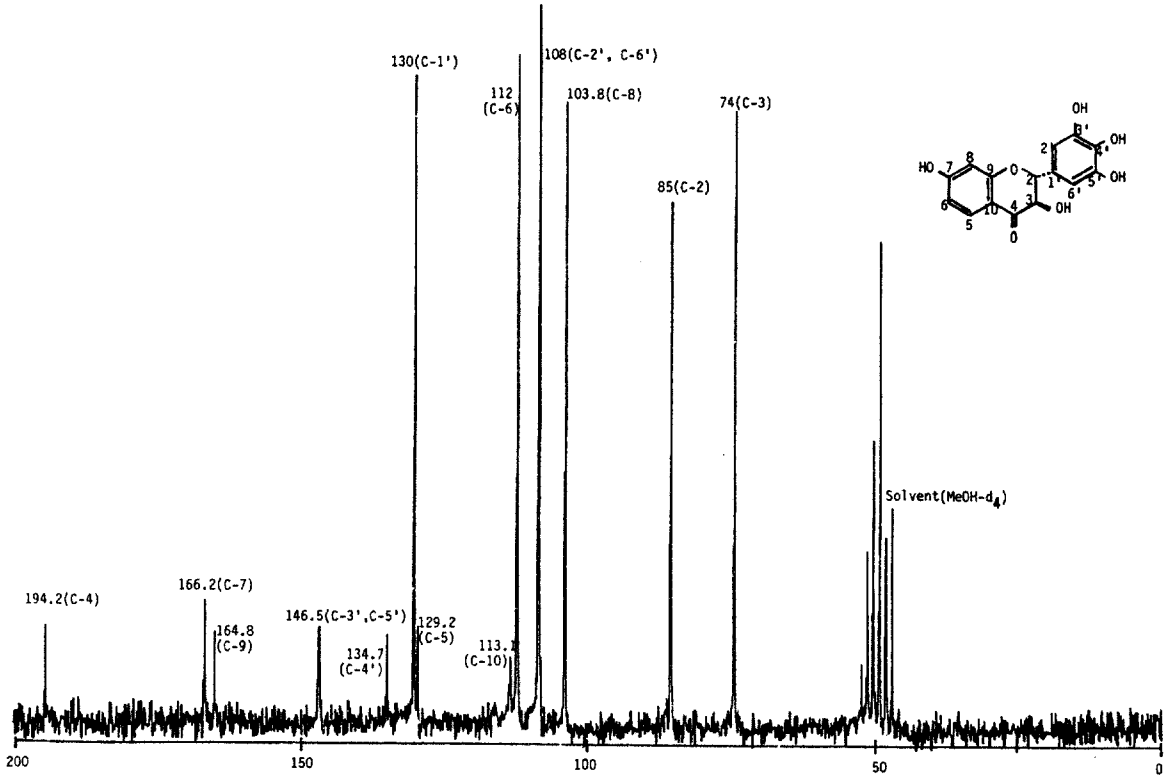

Fig. 3. ^{13}C NMR spectrum of compound 2.

4. EVALUATION

The yield of MeOH extraction of *Robinia pseudacacia* L. was 2%(42g) based on the air dried wood weight(2.1Kg).

This crude extractive gave 5.2g of water soluble fraction and 15.2g of EtOAc soluble materials which gave 4.9g of hexane soluble waxy materials, and the rest was water- and EtOAc-insoluble precipitate.

According to the results of this work, the extraction yield is very low and the extractive does not contain any reasonable amount of procyanidins which can be used for making of wood adhesive.

However, further investigation such as sulfonation of the extractive followed by isolation may be required to evaluate a potential for wood adhesive formulation using its bark extractive as well as wood extractive of the tree in the future.

In addition to the above, the EtOAc soluble

fraction of the extractive contains a large amount of (+)-dihydrorobinetin(ca. 25% of EtOAc soluble fraction) which is known to play an important role in the mechanisms of wood defenses due to its high decay and insect resistance.

Thus, *Robinia pseudacacia* L. should be reestimated in the point of full tree utilization such as board making or wood preservation using chemical or preservative properties of its chemical components.

5. CONCLUSIONS

The EtOAc soluble flavonoids of MeOH extractive of *Robinia pseudacacia* L. were isolated by column chromatography over Sephadex LH-20 using EtOH and MeOH-H_2O(1 : 1 and 1 : 3) as eluting solvents.

Two flavanoids were purified and isolated from the fraction and their structures were determined by TLC and NMR spectroscopy.

The isolated compounds were identified as (−)-butin, 7, 3′, 4′-trihydorxy flavanone, and (+)-dihydrorobinetin, 3, 7, 3′, 4′, 5′ -penta-hydroxy flavanonol.

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