論文（Original Article）

# Flavones from *Alnus rubra* Bong. seed coat

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**Abstract**

Flavonoid-like compounds are supposed to be signal compounds released from *Alnus rubra* to induce nitrogen-fixing nodule formation as a result of the symbiosis of *A. rubra* and *Frankia* spp. (Actinomycetales). However, there are no reports of flavonoid isolated from this plant except for tannins. Therefore, we have investigated the chemical constituents of *A. rubra*, especially those of the seeds coats, which could affect the nodulation in the early stage of plant growth, and the ability of the plant to thrive. As a result, five flavones, 7,4'-dimethoxy-5-hydroxyflavone (**1**), pectolinaringenin (**2**), acacetin (**3**), salvigenin (**4**), and apigenin (**5**) were isolated and their structures were determined by spectroscopic analyses. In the process, the previously reported mis-assigned $^{1}$H NMR chemical shifts for the two methoxyl groups of pectolinaringenin (**2**) were corrected based on 2D NMR experiments.

**Key words** : *Alnus rubra*, seed, flavone, 7,4'-dimethoxy-5-hydroxyflavone, pectolinaringenin, acacetin, salvigenin, apigenin

## INTRODUCTION

*Alnus rubra* (red alder) is a pacific North American deciduous tree that forms dense stands in the initial stage of primary succession on floodplains. Symbiosis with nitrogen-fixing Actinomycetes enhance the supply of available nitrogen in the soil, so it is suitable as a temporary nurse species for shade-tolerant conifers on nitrogen-deficient sites (Klinka et al., 1989).

Nitrogen-fixing nodule formation is well investigated in the symbiosis system of *Rhizobium* spp. bacteria and Leguminosae plants. The nodulation starts with contact of *Rhizobium* and the plant root, followed by bacterium induced altered growth of the epidermal hairs on the root, resulting in deformation or curling of the hair. The curled root hair traps bacteria and the bacteria start to proliferate, infecting outer plant cells and stimulating the production of the cell wall sheath known as "infection threads." This leads to nodule formation and further proliferation of the bacteria and ramification of the infection threads, where bacteria enveloped by the plant plasma membrane are released into plant cytoplasm and start symbiotic nitrogen fixation (Long, 1989).

In this process, induction of nodulation (*nod*) genes of *Rhizobium* bacteria is important for further nodule and symbiosis formation. Three flavones obtained from aqueous washing of seedlings of white clover (*Trifolium repens*), 7,4'-dihydroxyflavone, 7,4'-dihydroxy-3'-methoxyflavone (geraldone), and 4'-hydroxy-7-methoxyflavone were reported to induce *nod* genes in *Rhizobium trifolii* (Redmond et al., 1986). A flavone, 5,7,3',4'-tetrahydroxyflavone (luteolin) was obtained from alfalfa (*Medicago sativa*) seeds exudate as the active principle of *nod* gene inducing activity in *Rhizobium meliloti*. Two isoflavones, 7,4'-dihydroxyisoflavone (daizein) and 5,7,4'-trihydroxy-isoflavone (genistein) with *nod* gene inducing activities for *Bradyrhizobium japonicum* were isolated from methanol-water extract of soybean (*Glycine max*) seedlings (Kosslake et al., 1987).

However, although similar root hair deformation leading to nodule formation in symbiosis between *Frankia* spp. (Actinomycetales) filamentous prokaryotes and *A. rubra* has been reported (Berry et al., 1990), there are no reports about the identification of compounds with nodulation enhancing activity. Although, Beniot and Berry (Benoit et al., 1997) reported the existence of flavonoid-like compounds in the aqueous seed wash of *A. rubra*, they did not achieve the identification of those compounds. Therefore, we investigated the flavonoid constituents of the seed coat and seed of *A. rubra* and report here the results of isolation and identification of five flavones. As for the chemical constituent of *A. rubra*, although diarylheptanoids (Chen et al., 2000; Chen et al., 1998; Gonzalez-Laredo et al., 1998; Karchesy et al., 1974; Saxena et al., 1995),

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triterpenoids (Jain et al., 1971), and condensed tannnins (Karchesy et al., 1976) are reported from the bark of the plant, there are no reports of flavones obtained from this plant.

## EXPERIMENTAL

General. TLC was carried out using Merck pre-coated silica gel 60 plates (F254; layer thickness 0.20 for analytical TLC and 0.25 mm for prep. TLC). Compounds on the TLC plates were detected under UV light (wave length 365 nm and 254 nm) and with Gibbs reagent (Krebs et al., 1969; Tahara et al., 1984). EIMS spectrometry was carried out on a Kratos MS 50 instrument (direct insertion probe, 70 eV ionization potential). $^{1}$H NMR (200, 300 and 500 MHz) spectra were determined on a Bruker AC-200E, a Varian XL-300 and a Bruker AMX-500 respectively. $^{13}$C NMR (60 and 125 MHz) spectra were determined on a Varian XL-300 and a Bruker AMX-500 respectively. NOESY was recorded on JEOL α-500. TMS as an internal standard. Data are described in δ ppm.

*Plant materials and isolation of flavones. Alnus rubra* cones were collected in May 1998 in the campus of the University of British Columbia (voucher specimens deposited in the UBC herbarium). The seeds were separated from the cones when the cones were crumpled weakly. In this process, small particles meshed through 1 mm square mesh were also collected. They consisted of grayish waxy substance that coated the surface of the seeds and small broken woody substances from the cones and seeds etc (Photo.1). The separated samples (cones; 1360 g, seeds; 105.3 g and small particles; 20.4 g in dry weight) were extracted with 12 l and 600, and 100 ml of MeOH respectively. The crude extracts from the seeds and small particles afforded 12.0 and 13.1 g of substance respectively after concentration under reduced pressure. The seed extract dissolved in 100 ml of MeOH was extracted two times with 200 ml and 100 ml of hexane respectively. The combined hexane extract yielded 2.49 g of greenish paste after concentration under reduced pressure. The MeOH layer was concentrated under reduced pressure followed by organic solvent extraction using 200 ml of ethyl acetate and 100 ml of water two times. A brown paste (5.67 g) was obtained from the ethyl acetate extract after concentration under reduced pressure. The amount of water layer after concentration was 3.69 g. The small particle extract dissolved in 100 ml of MeOH was extracted with 200 ml and 100 ml of hexane respectively. The combined hexane extract yielded 3.34 g of greenish paste after concentration under reduced pressure. The MeOH layer was concentrated under reduced pressure followed by organic solvent extraction using 200 ml of ethyl acetate and 100 ml of water two times. A brown paste (6.15 g) was obtained from the ethyl acetate extract after concentration under reduced pressure. The amount of water layer after concentration was 1.30 g.

Photo.1. Seeds and small particles from seeds and cones used in this study (one scale; 1 mm).

The ethyl acetate fraction of the small particles was fractionated into 24 fractions by silica gel (120 g) column chromatography (Fr01; Hexane [H] : ethyl acetate [E] = 20 : 1, 500 ml, Fr02; H:E = 20 : 1; 300 ml, Fr03 - Fr05; H : E = 10 : 1; 300 ml each, Fr06 - Fr10; H : E = 5 : 1; 300 ml each, Fr11 - Fr 14; H : E = 3 : 1; 300 ml, Fr15 - Fr17; H : E = 1 : 1; 300 ml each, Fr18 - Fr20; ethyl acetate; 300 ml each, F21 and Fr 22; MeOH; 300 ml each, Fr23 and Fr 24 ; 1% acetic acid in MeOH; 300 ml each).

A Combined fraction of Fr08 and Fr09 denoted as Fr0809 (378.7 mg) was fractionated into 48 fractions by silica gel (12g) column chromatography (Fr0809-01 - Fr0809-16; H : chloroform [C] = 1 : 1, Fr0809-17 - Fr0809-26; H : C : methanol [M] = 50 : 50 : 1, Fr0809-27 - Fr0809-36; H : C : M = 50 : 50 : 3, Fr0809-37 - Fr0809-48; H : C : M = 50 : 50 : 6, eluate volumes were 10 ml respectively). Compound **1** was obtained (3.1 mg) from a combined fraction of Fr0809-17 to Fr0809-19 (denoted as Fr0809-1719) as pale yellow needles.

Pale yellow crystals formed in Fr14 were recrystalized from acetone and then from benzene-MeOH and 13.6 mg of compound **2** was obtained as needles. The pale yellow crystals formed in Fr 15 was recrystalized from acetone and 12.9 mg of compound **3** was obtained as needles.

The white sediment formed in the MeOH solution of Fr13 was separated. The mother liquids of Fr13, Fr14, and Fr15 were combined and denoted as Fr1315 (1.07g). This fraction was fractionated into 72 fractions silica gel (20 g) column chromatography (Fr1315-01 - Fr1315-15; H : C = 1 : 1, Fr1315-16 - Fr1315-22; H : C : M = 100 : 100 : 1, Fr1315-23 - Fr1315-36; H : C : M = 100 : 100 : 2, Fr1315-37 - Fr1315-50; H : C : M = 100 : 100 : 5, Fr1315-51 - Fr1315-62; H : C : M = 100 : 100 : 10, Fr1315-63 - Fr1315-72; H : C : M = 100 : 100 : 20, eluate volumes were 10 ml respectively). A combined fraction of Fr1315-21 - Fr1315-25 (denoted as Fr1315-2125, 99.7 mg) was subjected to preparative TLC using H : C : M = 5 : 15 : 4 as a developing solvent. As a

result compound **2** (10.8 mg) was again obtained from a band at *Rf* 0.36 which shows natural yellow on the TLC plate. A substance was obtained from a bond at *Rf* 0.15 that showed only quenching of a fluorescence on the TLC plate under a short wave length UV light, and was further subjected to a prep. TLC using H : diethyl ether : M : ammonia water = 30 : 50 : 4 : 1. Compound **4** (5.8 mg) was obtained from a band at *Rf* 0.36 as pale yellow needles.

A white sediment formed in MeOH : EtOAC = 1: 1 solution of a combined fraction of Fr16 and Fr17 denoted as Fr1617 (736.8 mg) was separated and the remaining substance (686.2 mg) in the mother liquid (denoted as Fr1617M) was fractionated into 72 fractions by silica gel (12g) column chromatography (Fr1617-01 - Fr1617-17; H : C = 1 : 1, Fr1617-18 - Fr1617-31; H : C : M = 100 : 100 : 2, Fr1617-32 - Fr1617-45; H : C : M = 100 : 100 : 5, Fr1617-46 - Fr1617-60; H : C : M = 100 : 100 : 10, Fr1617-61 - Fr1617-72; H : C : M = 100 : 100 : 20, eluate volumes were 10 ml respectively). Compound **5** (22.1 mg) was obtained from the combined fractions of Fr1617-57 - Fr1617-72 as pale yellow precipitate.

Physicochemical properties. 7,4'-dimethoxy-5-hydroxy-flavone (**1**). Gibbs test: slow blue.

EIMS m/z (rel. int.): 299 (19), 298 $[M]^+$ (100), 297 (11), 269 (16), 166 (8), 135 (14), 132 (10). $^1$H NMR (200 MHz, $CDCl_3$): δ 3.90 (6H, *s*, 7 and 4'-OMe), 6.35 (1H, *d, J* = ca 2 Hz, H-6), 6.43 (1H, *d, J* = ca 2 Hz, H-8), 6.58 (1H *s*, H-3), 7.00 (2H, *d, J* = ca 9 Hz, H-3', H-5'), 7.82 (2H, *d, J* = ca 9 Hz, H-2', H-6'). These data are in good agreement with literature values (Dhar et al., 1970; Silva et al., 1971).

*Pectolinaringenin* (**2**). Gibbs test: slow blue. EIMS *m/z* (rel. int.): 315 (26), 314 $[M]^+$ (100), 299 (64), 296 (42), 271 (28), 167 (13), 139 (14), 135 (11), 133 (28). $^1$H NMR (500 MHz, DMSO-*d6*): δ 3.75 (3H, *s*, 6-OMe), 3.84 (3H, *s*, 4'-OMe), 6.59 (1H, *s*, H-8), 6.83 (H, *s*, H-3), 7.08 (2H, *d, J* = 8.9 Hz, H-3', H-5'), 8.00 (2H, *d, J* = 8.9 Hz, H-2', H-6'), 10.67 (1H, *s*, 7-OH), 13.01 (1H, *s*, 5-OH). $^{13}$C NMR (125 MHz, DMSO-*d6*): δ 55.47 (4'-OMe), 59.87 (6-OMe), 94.23 (C-8), 103.00 (C-3), 104.10 (C-10), 114.50 (C-3', C-5'), 112.82 (C-1'), 128.21 (C-2', C-6'), 131.34 (C-2, 6), 157.4 (C-6), 152.35 (C-9), 152.92 (C-5'), 157.23 (C-7), 162.24 (C-4'), 163.30 (C-2), 182.08 (C-4).$^1$H NMR and $^{13}$C chemical shift data are in good agreement with literature values (Hase et al., 1995) except for the assignment of chemical shifts for the two methoxyl groups.

*Acacetin* (**3**). Gibbs test: slow blue. EIMS *m/z* (rel. int.): 285 (18), 284 $[M]^+$ (100), 283 (6), 241 (10), 152 (9), 133 (7), 132 (25). $^1$H NMR (200 MHz, Acetone-*d6*): δ 3.90 (3H, *s*, 4'-OMe), 6.25 (1H, *d, J* = ca 2 Hz, H-6), 6.55 (1H, *d, J* = ca 2 Hz, H-8), 6.70 (1H *s*, H-3), 7.15 (2H, *d, J* = ca 9 Hz, H-3', H-5'), 8.05 (2H, *d, J* = ca 9 Hz, H-2', H-6'), 13.00 (1H, *s*, 5-OH). These data are in good agreement with literature values (Duan et al., 1998).

*Salvigenin* (**4**). Gibbs test: slow blue. EIMS *m/z* (rel. int.): 329 (20), 328 $[M]^+$ (100), 327 (19), 313 (80), 299 (17), 285 (15), 282 (14). $^1$H NMR (300 MHz, Acetone-*d6*) : δ 3.79 (3H, *s*, 4'-OMe), 3.91 (3H, *s*, 6-OMe), 3.98 (3H, *s*, 7-OMe), 6.72 (1H, *s*, H-8), 6.87 (H, s, H-3), 7.13 (2H, *d, J* = 9 Hz, H-3', H-5'), 8.05 (2H, *d, J* = 9 Hz, H-2', H-6'), 10.67 (1H, *s*, 7-OH), 13.01 (1H, *s*, 5-OH). $^1$H NMR (200 MHz, $CDCl_3$): δ 3.87 (3H, *s*, 4'- OMe), 3.90 (3H, *s*, 6-OMe), 3.95 (3H, *s*, 7-OMe), 6.53 (1H, *s*, H-8), 6.58 (H, *s*, H-3), 7.00 (2H, *d, J* = 9 Hz, H-3', H-5'), 7.82 (2H, *d, J* = 9 Hz, H-2', H-6'), 10.67 (1H, *s*, 7-OH), 13.01 (1H, *s*, 5-OH). $^1$H NMR data in $CDCl_3$ are in good agreement with literature values (Youssef et al., 1995).

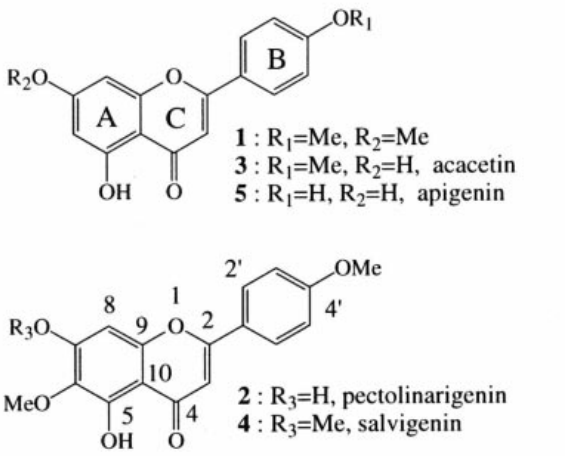


Fig. 1. Flavones isolated from *Alnus rubra* seed coat

*Apigenin* (**5**). Gibbs test: slow blue. EIMS *m/z* (rel. int.): 271 (16), 270 $[M]^+$ (100), 269 (14), 242 (10), 153 (13), 152 (10). $^1$H NMR (200 MHz, MeOH-*d4*): δ 6.20 (1H, *d, J* = ca 2 Hz, H-6), 6.45 (1H, *d, J* = ca 2 Hz, H-8), 6.55 (1H *s*, H-3), 6.95 (2H, *d, J* = ca 9 Hz, H-3', H-5'), 7.85 (2H, *d, J* = ca 9 Hz, H-2', H-6'). $^1$H NMR data are in good agreement with literature values (Loo et al., 1986).

Structures of the isolated flavones were shown in Fig. 1.

## RESULTS AND DISCUSSION

From the EtOAc fraction of the MeOH extract of small particles of *A. rubra* seeds, five compounds **1** - **5** were isolated. Compound **2** showed blue color slowly on TLC against Gibbs reagent suggesting the existence of a phenol group with a hydrogen bond (Tahara et al., 1984). $^{13}$C NMR showed 17 carbon signals including two methoxyl carbons ( 55.47 and 59.87 ppm), 14 aromatic or olefinic carbones, and a carbonyl (182.08 ppm) carbon, indicative of a flavone structure for compound **2**. $^1$H NMR showed a para-substituted phenyl ring system (7.08 ppm [2H, *d, J* = 8.9 Hz] and 8.00 pmm [2H, *d, J* = 8.9 Hz]), two aromatic or olefinic protons (6.59 and 6.83ppm) that appeared as singlets, and two hydroxyl group protons (10.67 and 13.01 ppm). The latter proton is assignable as a hydroxyl proton with a hydrogen bond and could be assigned as a hydroxyl group on C-5 position of a flavone compound. EIMS spectrum showed *m/z* 314 as a base ion and fragment ions of *m/z* 167 and 133,

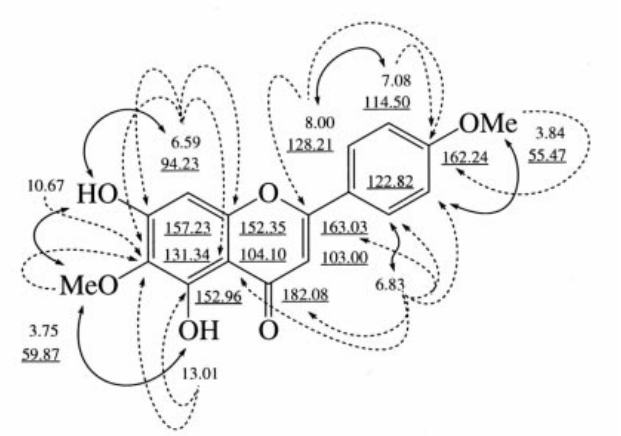


Fig. 2. C-H long range correlations (- - - ▶ ) and NOEs (◀——▶ ) found in HMBC and NOESY experiments respectively.

which could be obtained by a retro-Diels-Alder fission of a flavone with one methoxyl group and two hydroxyl groups on A ring (*m/z* 167) and one methoxyl group on B ring (*m/z* 133). Although, the two singlet protons found at 6.59 and 6.83 ppm could be assigned for the protons at C-8 and C-3 of a flavone compound, the position of a methoxyl group on A ring was not certain. In order to clarify the position of this methoxyl group and to assign all of the chemical shifts of the carbons, we carried out HSQC and HMBC experiments. The chemical shifts of all the protonated carbons were assigned firmly based on the cross peaks found in HSQC. Based on the C-H long range correlations found in the HMBC experiment, all the carbons were assigned as shown in Fig. 2 except for the C-7 and C-9 carbons which showed HMBC only with H-8. However, because there were several unambiguous assignments for those carbons in similar compounds based on 2D NMR experiments (Hanawa et al., 1991; Loo et al., 1986; Youssef et al., 1995), the chemical shifts of C-7 and C-9 of compound **2** were assigned as shown in Fig. 2. Therefore compound **2** was identified as Pectolinaringenin. However, because Hase et al (Hase et al., 1995) reported different $^1$H NMR chemical shift assignments for the 6-OMe (3.86 ppm) and 4'-OMe (3.75 ppm) of this compound based on their NOE experiment, which were opposite to our assignments, we carried out an NOESY experiment. As a result, NOE cross peaks are found between 6-OMe (3.75 ppm) and 5- and 7-OH (13.01 and 10.67 respectively), 4'-OMe (3.84 ppm) and H-3' (7.08 ppm), and others as shown in Fig. 2. This result supported our assignment given in Fig. 2. In addition, although Hase's group did not detect the NOEs between 6-OMe and 5- and 7-OH, an NOE was reported between 6-OMe and 5-OH of salvigenin (**4**) (Youssef et al., 1995) as we did in compound **2**. Therefore there might be something wrong with Hase's experiment. Moreover, a reported chemical shift assignment for 4'-OMe (3.89 ppm) of acacetin (**3**) in DMSO-*d6* (Duan et al., 1998) is much closer to 3.86 ppm than 3.75 ppm obtained in our experiment (in DMSO-*d6*) for the two methoxyl groups of pectolinaringenin (**2**). This result also supports our assignments.

The other compounds isolated from *A. rubra* were identified as 7,4'-dimethoxy-5-hydroxyflavone (**1**), acacetin (**3**), salvigenin (**4**), and pigenin (**5**) based on the spectroscopic analysis and on the comparison of the data with literature values.

As mentioned above, we isolated five flavones from the small particles consist of waxy substance from the seeds and the small broken woody substances from the cones and seeds etc (Photo.1). Some of those flavones could be potential nodulation inducers that are involved in the *Frankia* spp. and *A. rubra* symbiosis system. Apigenin (**5**) isolated in this experiment is reported to induce the *nod* gene in alfalfa and *Rhizobium* system (Firmin et al., 1986; Peters et al., 1986). Therefore, some of the flavones reported here could be the unidentified active nodulation inducing principles in Frankia spp. and *A. rubra* symbiosis (Benoit et al., 1997). Some of the flavones isolated in this experiment might have a negative effect on the nodulation of *Frankia* and *A. rubra* symbiosis as in the case of some flavonoids which inhibit the *nod* gene inducing activities of other flavonoids in pea and *Rhizobium* (Firmin et al., 1986) or alfalfa and *Rhizobium* (Peters et al., 1988) systems.

*Nod* gene inducing compounds in the seed or seed coat are supposed to be important for the symbiosis of alfalfa and *Rhizobium*. An alfalfa cultivar seedling with a higher amount of luteolin showed higher $N_2$ fixation than a cultivar with a lower amount of luteolin (Kapulnik et al., 1987), and when luteolin was added to the rhizhosphere of the latter cultivar, the $N_2$ fixation and total dry weight of the plant increased. During 4 hours of imbibition, the total *nod* gene-inducing activity released from the seed was at 100-fold higher rates than from the roots of 72-hour-old seedlings of alfalfa (Hartwig et al., 1990). The primary source of this activity was determined to be the seed coats which contained luteolin and luteolin-7-*O*-glucoside which could be used as a *nod* gene inducer after hydrolysis by the glucosidase activity released from the plant during the first 4 hours of imbibition (Hartwig et al., 1991). Therefore the flavones isolated from the seed coat of *A. rubra* could be a good source of nodulation signals which were important for the early growth of the plant.

Although we obtained the small particles that consist of waxy substance and small broken woody substances used in this experiment in the separation process of seed from cones (Photo. 1), the possibility of some contamination of those particles was not completely denied. Therefore we compared the constituents of the MeOH extract of the seeds and those of the small particles and confirmed that they have the same constituents. As shown in Fig. 3, the TLC profile of the EtOAc fractions of the MeOH extract of seeds and small particles are identical. There are three spots in both fractions that showed

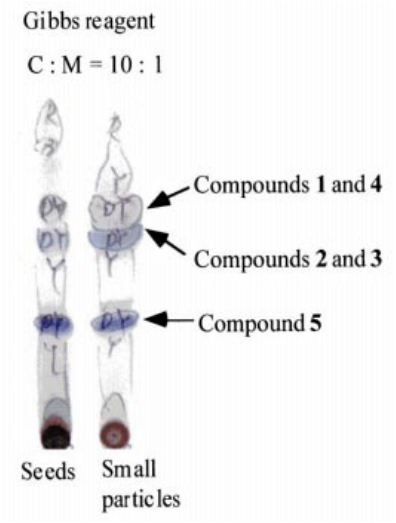


Fig. 3. Comparison of the constituents in the EtOAc fractions from MeOH extracts of *A. rubra* seeds (left) and small particles (right). Five ml of sample from each EtOAc fraction adjusted to the concentration 1 g / ml were charged on TLC and developed at room temperature using chloroform : MeOH = 10 : 1 as a developing solvent. Phenolic compounds that do not have any substitution at the *para* position of the phenol group reacted with Gibbs reagent.

coloring reactions with Gibbs reagent that react with the unsubstituted phenol group at the *para* position. Furthermore microscopic examination on the surface of the seed revealed the existence of wax-like substances similar to the wax-like substances in the small particles. Therefore we concluded that the small particles consist of substances mainly derived from the seeds. The compounds in the three spots from the top to the bottom of the TLC plate in Fig. 3 are considered to be the flavones with one hydroxyl group (compound **1** and **4**), two hydroxyl groups (compound **2** and **3**) and three hydroxyl group (compound **5**) respectively.

The amount of organic solvent extractable constituents in the small particles is worth to mention. We obtained 13.1 g of crude extract from only 20.4 g of small particles (64 % of the total weight was extractable with organic solvent). In contrast, 12.0 g of crude extract was obtained from 105.3 g of seeds (extractable portion was only 11 % of the total weight). Therefore the small particles are a good source of flavones which are thought to play an important role in *Frankia* and *A. rubra* symbiosis. They could scatter in the soil when the seeds falls from the cones and could provide the above mentioned flavones in the soil that might be able to activate the nodulation gene of *Frankia* near the seeds.

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# レッドアルダー（*Alrus rubra* Bong.）種子表皮物質由来フラボンの単離・構造決定

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要　旨

ハンノキ科植物である*Alnus rubra*より放出されるフラボノイド様化合物が、*A. rubra*とフランキア属放線菌の共生関係による窒素固定に関与するシグナル物質であることが示唆されている。しかしながら、この植物からの単離されているフラボノイドに関しては、タンニンの報告が一例有るのみである。我々は、*A. rubra*の化学成分中のフラボノイドに興味を持ち、その中でも特に本植物の成長初期の根瘤形成に大きな影響を持つと考えられる本植物の種子および種子表皮物質中の二次代謝産物について調査を行った。その結果、５つのフェノール性化合物を単離し、核磁気共鳴スペクトルや質量分析等のスペクトル解析によりその構造を 7, 4'-ジメトキシ-5-ヒドロキシフラボン (7, 4'-dimethoxy-5-hydroxyflavone) (**1**)、ペクトリナリンゲニン (pectolinaringenin) (**2**)、アカセチン (acacetin) (**3**)、サルビゲニン(salvigenin) (**4**)、およびアピゲニン (apigenin) (**5**) と決定した。この過程において、過去の報告にある pectolinaringenin (**2**) が持つ２つのメトキシル基のプロトン NMR における化学シフトの帰属が誤りであることを、二次元 NMR の詳細な解析により明らかにし、新たな帰属を提案した。

キーワード：*Alnus rubra*、種子、フラボン、7, 4'-ジメトキシ-5-ヒドロキシフラボン (7, 4'-dimethoxy-5-hydroxyflavone)、ペクトリナリンゲニン (pectolinaringenin)、アカセチン (acacetin)、サルビゲニン (salvigenin)、アピゲニン (apigenin)

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