Chemical Constituents of *Alnus sieboldiana* (BETULACEAE)[1] II. The Isolation and Structure of Flavonoids and Stilbenes

Yoshinori Asakawa

Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Hiroshima

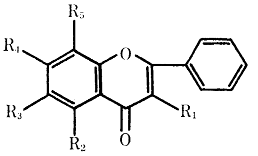
(Received March 18, 1971)

Four new flavonoids with an unsubstituted B-ring, alnusin, alnetin, alnustin, and alnustinol, isolated from *Alnus sieboldiana* were established to be 3,5,7-trihydroxy-6-methoxyflavone (**1**), 5-hydroxy-6,7,8-trimethoxyflavone (**2**), 5-hydroxy-3,6,7-trimethoxyflavone (**3**), and 3,5,7-trihydroxy-6-methoxyflavanone (**15**) respectively. Some previously-known flavonoids, chrysin (**4**), izalpinin (**5**), tectochrysin (**6**), pinocembrin (**16**), pinobanksin (**17**), strobopinin (**19**) and naringenin (**20**), and some stilbenes, pinosylvin (**24**), pinosylvin monomethyl ether (**25**), and pinosylvin dimethyl ether (**26**), were identified along with the above new flavonoids.

In previous papers,[2] we have reported the isolation and the structure of two new hydroxyketones, *trans*-stilbene (**23**), pinostrobin (**18**), alpinetin (**21**), β-phenylethyl cinnamate, and cinnamic acid from the benzene extract of the male flower of *Alnus sieboldiana.* In the course of continuing the investigation on the viscous substance of the male flower, we further isolated four new flavonoids,[3] accompanied by some previously-known flavonoids and stilbenes. We now wish to report on the structural elucidation, and to present our supposition as to the biosynthetic pathway, of these compounds.

Results and Discussion

The isolation of flavonoids and stilbenes was performed by utilizing their solubility difference in ether and methanol, followed by a combination of column- and preparative thin-layer chromatography on silica gel.



Alnusin(1)	$R_1=R_2=R_4=OH$　$R_3=OMe$　$R_5=H$
Alnetin(2)	$R_1=H$　$R_2=OH$　$R_3=R_4=R_5=OMe$
Alnustin(3)	$R_1=R_3=R_4=OMe$　$R_2=OH$　$R_5=H$
Chrysin(4)	$R_1=R_3=R_5=H$　$R_2=R_4=OH$
Izalpinin(5)	$R_1=R_2=OH$　$R_3=R_5=H$　$R_4=OMe$
Tectochrysin(6)	$R_1=R_3=R_5=H$　$R_2=OH$　$R_4=OMe$
(7)	$R_1=R_2=R_4=OAc$　$R_3=OMe$　$R_5=H$
(8)	$R_1=R_3=R_4=OMe$　$R_2=OH$　$R_5=H$
(9)	$R_1=R_2=R_3=R_4=OMe$　$R_5=H$
(10)	$R_1=R_2=R_4=OEt$　$R_3=OMe$　$R_5=H$
(11)	$R_1=H$　$R_2=R_3=R_4=R_5=OMe$

Fig. 1

Alnusin (1). Alnusin, the main flavonoid of the plant, was crystallized as yellow plates, mp 239—241°C. Its elemental analysis and the mass spectrum indicated the formula $C_{16}H_{12}O_6$. The compound was shown to be a flavone by alcoholic ferric chloride and magnesium hydrochloric acid tests. The IR spectrum indicated the presence of an α,β-unsaturated carbonyl group (1640 cm^{-1}). The presence of hydroxyl groups were confirmed by the infrared absorption bands at 3350 and 3300 cm^{-1} and by the NMR signal of two singlets at 9.25 and 12.23 ppm. The signal in the far-down field showed the presence of a hydroxyl group which is hydrogen-bonded to the carbonyl group. The acetylation of **1** with acetic anhydride gave the triacetate (**7**), confirming the presence of three hydroxyl groups. The absence of *o*-dihydroxyl groups was indicated by the lack of any appreciable change in the UV spectrum of alnusin on the addition of boric acid-sodium acetate.[4] On the other hand, the presence of the C_7–OH group was suggested by the bathochromic shift on the addition of sodium acetate to the alcoholic solution of **1**.[4] The presence of C_5–OH group was shown by the bathochromic shift in the UV spectrum on the addition of aluminum chloride[4] and by the NMR spectrum of the triacetate, which showed the characteristic resonance of C_5–OAc as a singlet at 2.48 ppm, unlike those of other flavone acetyl groups.[5] The presence of a monosubstituted benzene-ring was shown by the infrared absorption bands at 762 and 680 cm^{-1} and by the A_2B_3 signals of the NMR spectrum at 7.60 and 8.20 ppm. The NMR spectrum of **1** showed a signal for one methoxy proton at 3.89 ppm. The position of the methoxy group was confirmed to be C_6 by the intense quinoid cation (M-15, *m/e* 285) of the mass spectrum, which is characteristic of flavones with the C_6–OMe group.[6] The one-proton singlet at 6.88 ppm was assinged to either the C_3- or the C_8-proton. The treatment of **1** with diazomethane gave trimethylether (**8**), while methylation with dimethyl sulfate resulted in 3,5,6,7-tetramethoxyflavone (**9**).[7] The oxygenation pattern was established by the alkaline degradation of **9** which gave 2-hydroxy-ω-4,5,6-

---

[1] The plant name "*Alnus firma* Sieb. et Zucc." has been given in our previous papers.[2] However, we now wish to correct it to "*Alnus sieboldiana,*" due to the identification of *Alnus* species by Professor Hyoji Suzuki of Hiroshima University.

[2] a) Y. Asakawa, F. Genjida, S. Hayashi, and T. Matsuura, *Tetrahedron Lett.*, **1969**, 3235. b) Y. Asakawa, This Bulletin, **43**, 575 (1970). c) Y. Asakawa, *ibid.*, **43**, 2223 (1970).

[3] Y. Asakawa, F. Genjida, and T. Suga, This Bulletin, **44**, 297 (1971).

[4] L. Jurd, "The Chemistry of Flavonoid Compounds," ed. by T. A. Geissman, Pergamon Press, New York (1962),p. 107.

[5] C. A. Henrick and P. R. Jefferies, *Aust. J. Chem.*, **17**, 934 (1964).

[6] J. H. Bowie and D. W. Cameron, *Aust. J. Chem.*, **19**, 1627 (1966).

[7] A. C. Jain, T. R. Seshadri, and K. R. Sreenivasan, *J. Chem. Soc.*, **1955**, 3908.

tetramethoxyacetophenone (**12**) and benzoic acid (**14**). Thus, the structure of alnusin is undoubtedly 3,5,7-trihydroxy-6-methoxyflavone. The NMR solvent shift data[8] obtained on changing the solvent from deuteriochloroform to benzene for alnusin triethylether (**10**) offered further support for the structure of alnusin.

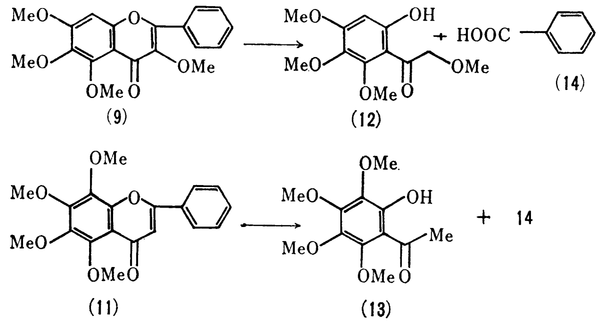
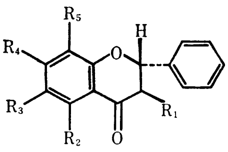
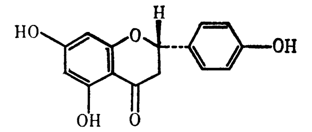


Fig 2.

Alnetin (2). Alnetin was obtained as pale yellow needles (mp 100—101°C). The elemental and mass spectral analyses supported the molecular formula of $C_{18}H_{16}O_6$ (M^+ 328). It showed a positive coloration with ferric chloride. The IR spectrum showed the presence of a hydroxyl group, an α,β-unsaturated carbonyl group, and a monosubstituted benzene-ring. The considerable bathochromic shift of the UV spectrum upon the addition of aluminum chloride to the alcoholic solution of **2** suggested the presence of the C_5–OH group, which was also supported by the NMR signal at 12.45 ppm (s, 1H). The absence of the C_7–OH and *o*-dihydroxyl groups was based on the lack of any appreciable change in the UV spectrum upon the addition of sodium acetate or boric acid-sodium acetate. The NMR spectrum indicated the presence of three methoxy groups (3.95, 3.97, 4.10 ppm, each s, 9H), one aromatic proton (6.69 ppm, s, 1H), monosubstituted benzene-ring protons (7.56, 7.95 ppm, m, 5H), and a chelated hydroxyl group (12.45 ppm, s, 1H). The methylation of **2** with dimethyl sulfate afforded a tetramethoxy derivative (**11**) which was identical with the 5,6,7,8-tetramethoxyflavone reported by Lee *et al.*[9] The hydrolysis of the tetramethoxy derivative (**11**) with aluminum chloride in ether gave the original compound. The treatment of **11** with a 50% potassium hydroxide solution gave 2-hydroxy-3,4,5,6-tetramethoxyacetophenone (**13**) and benzoic acid (**14**). Alnetin has thus been assinged the (**2**) structure. This compound has never been isolated from any natural source; it has been reported only as a synthetic product.[9]

Alnustin (3). Alnustin, $C_{18}H_{16}O_6$, mp 175—176°C, M^+ 328, was obtained as yellow plates. The compound showed a positive coloration with ferric chloride, and all of its spectra were typical of flavoniods. The IR spectum showed the presence of an α,β-unsaturated carbonyl group and a monosubstituted benzene-ring. The UV spectrum, upon the addition of sodium acetate, boric acid-sodium acetate, or aluminum chloride, showed that **3** contained no *o*-dihydroxyl groups and no C_7–OH, but it did contain the free C_5–OH group. The presence of the C_5–OH group was also indicated by the far-down signal at 12.58 ppm (s, 1H) in the NMR spectrum. The other signals in the spectrum were assigned to monosubstituted benzene-ring protons (7.56, 8.10 ppm, m, 5H), three methoxy groups (3.89, 3.94, 3.97 ppm, each s, 9H), and C_8–H (6.54 ppm, s, 1H). On methylation with dimethyl sulfate, Compound (**3**) gave a tetramethoxy derivative (**9**) which was identical with 3,5,6,7-tetramethoxyflavone. The demethylation of **9** with aluminum chloride in ether gave the original compound. Thus, the structure of alnustin was established as 5-hydroxy-3,6,7-trimethoxyflavone.





Naringenin (20)

Alnustinol(15) $R_1=R_2=R_4=OH$ $R_3=OMe$ $R_5=H$
Pinocembrin(16) $R_1=R_3=R_5=H$ $R_2=R_4=OH$
Pinobanksin(17) $R_1=R_2=R_4=OH$ $R_3=R_5=H$
Pinostrobin(18) $R_1=R_3=R_5=H$ $R_2=OH$ $R_4=OMe$
Strobopinin(19) $R_1=R_5=H$ $R_2=R_4=OH$ $R_3=Me$
Alpinetin(21) $R_1=R_3=R_5=H$ $R_2=OMe$ $R_4=OH$
(22) $R_1=R_2=OH$ $R_3=R_4=OMe$ $R_5=H$

Fig 3.

Alnustinol (15).[10] Alnustinol was isolated in a minute quantity as colorless needles, mp 175—176°C, M^+ 302. The compound gave the characteristic coloration of flavanonols with ferric chloride, magnesium hydrochloric acid, and zinc hydrochloric acid and in Pachcós tests. The IR spectrum indicated the presence of hydroxyl groups, an α,β-unsaturated carbonyl group, and a monosubstituted benzene-ring. The lack of the bathochromic shift in the UV spectrum, on the addition of boric acid-sodium acetate, showed the absence of *o*-dihydroxy groups. On the other hand, the considerable bathochromic shift on the addition of aluminum chloride or sodium acetate indicated the presence of the C_7–OH and C_5–OH groups. The latter hydroxyl group was also confirmed by the NMR signal at 11.34 ppm (s, 1H). The NMR spectrum exhibited the signals at 3.86 ppm (s, 3H) assignable to a methoxy group, at 4.40 ppm and 4.97 ppm (typical AB doublet, $J=12$ Hz, 2H) assignable to the flavanonol structure, and at 5.99 ppm (s, 1H) to C_8–H, and at 7.33 ppm (s, 5H) assignable to monosubstituted benzene-ring protons. The presence of the C_3–OH group was supported by the fragment ions at *m/e* 273 (M–CHO) and 91 (120–CHO) which are characteristic of 3-hydroxyflavanone.[11] Chemical evidence in favor

---

[8] R. G. Wilson, J. H. Bowie, and D. H. Williams, *Tetrahedron*, **24**, 1407 (1968).

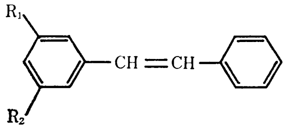
[9] H. H. Lee and C. H. Tan, *J. Chem. Soc.*, **1965**, 2743.

[10] In the previous paper,[3] the structure of alnustinol has been given as 3,5,8-trihydroxy-7-methoxyflavanone. The author now wishes to correct it to 3,5,7-trihydroxy-6-methoxyflavanone from the results of the reexamination of the solvent shift in the UV spectrum and the chemical reactions.

[11] H. Audier, *Bull. Soc. Chim. Fr.*, **1966**, 2892.

of the C_5–OH and C_3–OH groups in alnustinol was obtained by its reaction with diazomethane,[12] which gave only a monomethylated compound (**22**). Hence, the structure of 15 may be said to be 3,5,7-trihydroxy-6-methoxy- or 3,5,7-trihydroxy-8-methoxyflavanone. The dehydrogenation of **15** with palladium charcoal gave a flavonol which was shown to be identical with 3,5,7-trihydroxy-6-methoxyflavone (**1**). On the basis of the above chemical and spectral evidence, alnustinol was established to be 3,5,7-trihydroxy-6-methoxyflavanone (**15**). 3,5,6,7-Tetrasubstituted- and fully oxygenated A-ring flavonoids with an unsubstituted B-ring are rare in nature.

The Known Flavonoids and Stilbenes. Nine flavonoids, chrysin (**4**), izalpinin (**5**), tectochrysin (**6**), pinocembrin (**16**), pinobanksin (**17**), pinostrobin (**18**), strobopinin (**19**), naringenin (**20**), and alpinetin (**21**), were identified by the comparison of their physical and chemical properties with those of authentic samples or with those reported previously. Also, three stilbenes, pinosylvin (**24**), pinosylvin monomethyl ether (**25**), and pinosylvin dimethyl ether (**26**), were confirmed by the same methods as above. In the mass spectrum of *trans*-stilbene (**23**), the loss of the methyl radical (M-15) from the molecular ion is observed; this fragmentation process has shown, by the deuterium labelling, that one of the central CH groups, together with an ortho-hydrogen atom from either ring, is eliminated.[13] Similar losses of a methyl radical are also observed in pinosylvin, pinosylvin monomethyl ether, and pinosylvin dimethyl ether. Except for the M-15 ion, all these stilbenes involve the strong fragment ion at *m/e* 165 which was determined to be $C_{13}H_9^+$ by high-resolution mass measurements. Thus, stilbenes isolated from natural source can be detected by the mass spectrum with characteristic fragment ions at *m/e* M-15, and *m/e* 165 ($C_{13}H_9^+$), and the molecular ion appears as a base peak. It is known that the stilbenes of the genus Pinus serve as chemotaxonomic tracers in the *Pinaceae*[14] and that pinosylvin and its monomethyl ether have biological activity and protect the heartwood from wood-rotting fungi and insects.[15] Stilbenes have been found in *Pinaceae*, *Moraceae*, *Saxifragaceae*, *Myrtaceae*, *Polygonaceae*, *Fagaceae*, *Liguminosae*, and *Liliaceae* as free and/or glycoside forms.[16] Hence, it is notable that this paper is reporting the first example of the isolation of the stilbenes (**23**, **24**, **25**, and **26**) from the *Betulaceae*.



trans-Stilbene (23) $R_1=R_2=H$
Pinosylvin (24) $R_1=R_2=OH$
Pinosylvin monomethyl ether (25) $R_1=OH$ $R_2=OMe$
Pinosylvin dimethyl ether (26) $R_1=R_2=OMe$

Fig 4.

Biogenesis. Flavonoids and stilbenes have been found to occur together in the heartwood of various pines,[14] *Morus bomycis*,[17] and *Eucalyptus* species.[18] Apparently, the co-existence of these compounds has never been found in *Betulaceae*. The coexistence of the flavonoids and stilbenes in the same plant and the analogy of the structure of the isolated compounds provide important clues for the elucidation of their biogenesis. Almost all the compounds isolated from *A. sieboldiana* possessed a monosubstisuted benzene-ring in the molecule; only naringenin (**20**) did not. The male flower of this plant contained a large quantity of free cinnamic acid. Accordingly, the above monosubstituted benzene-ring must arise from cinnamic acid. The route of synthesizing pinosylvin (**24**) has recently been established by the isolation of the enzyme, which catalyzes the cyclization of cinnamoyl triacetic acid to pinosylvin (**24**), from *Eucalyptus sideroxylon* leaves,[18] while the formation of various flavonoids from chalkone has been confirmed by means of a parallel competetive feeding experiment.[19]

The flavonoids and stilbenes isolated from *A. sieboldiana* may be formed by a combination of the shikimic acid and acetate (polyketide) pathways. We would like to propose the biosynthetic pathway of the flavonoids and stilbenes shown in Scheme 1.

Experimental

All the melting points are uncorrected. Analytical and preparative tlc were carried out using silica gel GF_{254} and $HF_{254+366}$ (Merck) using a solvent composed of benzene, dioxane, and acetic acid (90:25:4 vol%). The spot was detected by using the UV light (254 and 366 nm) and/or iodine vapor. The mass spectra were recorded on a Hitachi-RMU 6D Mass spectrometer under the following conditions: chamber volt, 70 V; vacuum, 3.0×10^7 mmHg; ion chamber temp., 250°C; direct sample-introduction system. The high-resolution mass spectra were measured on a MS 902 double-focusing instrument. The UV, IR, and NMR spectra were measured by the methods reported in a preceding paper.[2c]

Extraction and Isolation. The *Alnus sieboldiana* was collected near Hiroshima City in March, 1969. The male flower (95 kg) was extracted with benzene for three months. The benzene extract (780 g) was successively extracted with a 5% aqueous sodium bicarbonate solution (Fraction 1), a 5% aqueous sodium carbonate solution (Fraction 2), and a 5% sodium hydroxide solution (Fraction 3).

Alnustinol (15). The neutralization of Fraction 1 with dilute hydrochloric acid and extraction with ether yielded a brown slurry (8 g). It was triturated with ether to give alnustinol (**15**). The crystallization of **15** from *n*-hexane gave colorless needles (58 mg); mp 175—176°C; $FeCl_3$ (dark

---

[12] R. Robinson and K. F. Tseng, *J. Chem. Soc.*, **1938**, 1004.

[13] R. A. W. Johnstone and B. J. Millard, *Z. Naturf.*, **21a**, 604 (1966).

[14] G. Lindstedt and A. Misiorny, *Acta Chem. Scand.*, **5**, 121 (1951).

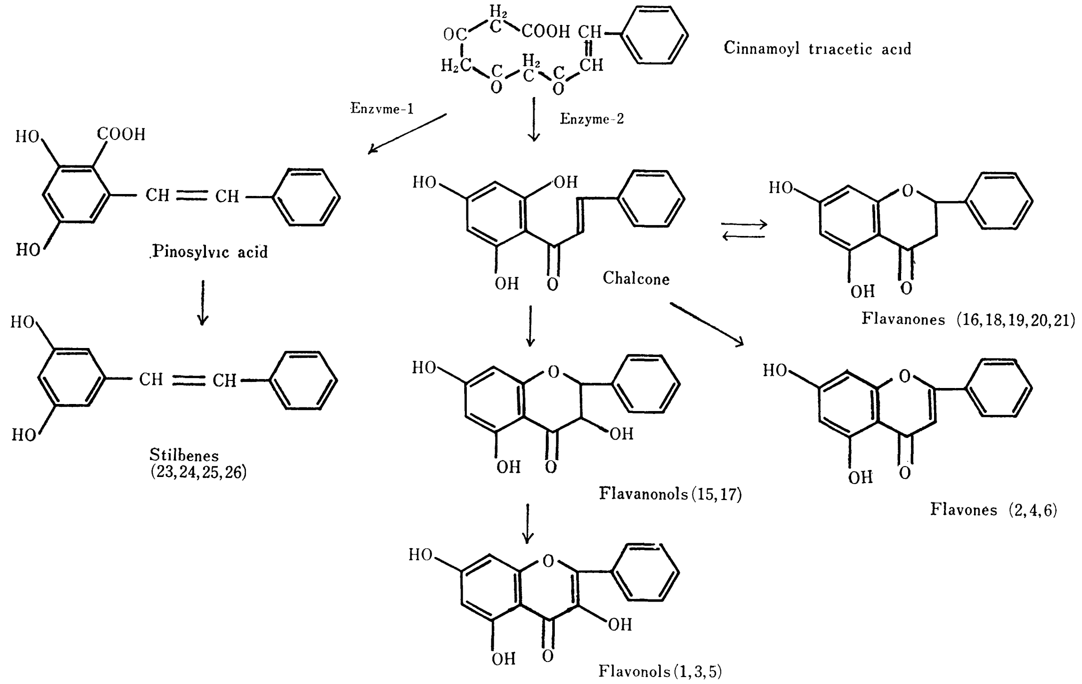
[15] H. Erdtman and E. Rennerfelt, *Svensk Pappersmasse Tidn*, **47**, 45 (1944).

[16] G. Billek, *Fortschr. Chem. org. Naturst.*, **22**, 115 (1964).

[17] T. Knodo, H. Ito, and M. Suda, *Nippon Nogeikagaku Kaishi*, **32**, 1 (1958).

[18] W. E. Hillis and N. Ishikura, *Phytochem.*, **8**, 1079 (1969).

[19] E. Wong, *Chem. Comm.*, **1968**, 395. E. Wong and H. Grisebach, *Phytochem.*, **8**, 1419 (1969).



Scheme 1. Biosynthetic pathways of flavonoids and stilbenes isolated from the male flower of *A. sieboldiana*.

green), Mg-HCl (yellow), Zn-HCl (yellow), Pachcós test (reddish brown); UV spectrum $\lambda_{\max}^{\mathrm{EtOH}}$ 215 nm (log ε, 4.58), 226_{sh} (4.48), 295 (4.32), 340 (3.65), $\lambda_{\max}^{\mathrm{EtOH+AlCl_3}}$ 226 (4.36), 318 (4.48), 400 (3.55), $\nu_{\max}^{\mathrm{EtOH+NaOAc}}$ 216 (4.34), 300 (4.14), 335 (4.41), $\lambda_{\max}^{\mathrm{EtOH+NaOEt}}$ 215 (4.43), 245 (4.18), 333 (4.41); IR spectrum, $\lambda_{\max}^{\mathrm{Nujol}}$ 3480, 3460, 3340 (OH), 1635—1654 (CO), 1588, 1498, 1358, 1298, 1175, 1164, 1126, 1086, 1000, 990, 963, 884, 838, 797, 752, 685 cm^{-1}; NMR signals ($CDCl_3$), δ=3.49 (s, C_3–OH), 3.86 (s, C_6–OMe), 4.40 (d, J=12 Hz, C_3–H), 4.97 (d, J=12 Hz, C_2–H), 5.99 (s, C_8–H), 7.33 (s, 5H, B-ring protons), 11.34 ppm (s, C_5–OH); mass spectrum (prominent) *m/e* 302 (M^+), 273, 195, 183 (base), 182, 167, 156, 120, 91, 77, 69.

Found: C, 63.95; H, 4.87%. Calcd for $C_{16}H_{14}O_6$; C, 63.57; H, 4.67%.

*Chrysin (**4**), Pinocembrin (**16**),* and *pinobanksin (**17**).* Fraction 2 was acidified with 5% hydrochloric acid and then extracted with chloroform. The refrigeration of the brown viscous material (0.85 g) dissolved in ether diposited a yellow mass. Subsequent crystallization from methanol gave chrysin (**4**) (40 mg); mp 275—277°C, lit,[20] 278—279°C; $\nu_{\max}^{\mathrm{Nujol}}$ 3350—3050 (OH), 1640 (CO), 795 cm^{-1}; *m/e* 254 (M^+, base), 226, 152, 124, 113, 77, 69. On the other hand, more cooling of the mother liquor gave another pale yellow mass. Subsequent crystallization from methanol afforded white needles of pinocembrin (**16**) (150 mg); mp 200—201°C, lit,[21] 193—194°C; $\nu_{\max}^{\mathrm{Nujol}}$ 3250—2500 (OH), 1623 (CO), 1155 cm^{-1}; *m/e* 256 (M^+, base), 179, 153, 152, 124, 104, 103, 96, 78, 77, 69, 51. When the combined mother liquor was subjected to preparative thin-layer chromatography, it yielded pinobanksin (**17**) (8 mg); mp 177—178°C, lit,[21] 177—178°C; $\nu_{\max}^{\mathrm{Nujol}}$ 3465 (OH), 1625 (CO), 815 cm^{-1}.

*Alnusin (**1**), Alnetin (**2**), Alnustin (**3**), Tectochrysin (**6**), Izalpinin (**5**), Pinostrobin (**18**), Strobopinin (**19**), Naringenin (**20**), Alpinetin (**21**), Pinosylvin (**24**),* and *Pinosylvin monomethyl ether (**25**).* Fraction 3 was extracted with chloroform after acidification with dilute hydrochloric acid. The removal of the solvent yielded a brown solid (8.45 g), which was then dissolved in ether and kept in a refrigerator. The major flavonol, alnusin (**1**), was thus deposited as yellow mass (2.85 g); mp 239—241°C (from methanol); $FeCl_3$ (dark green), Mg-HCl (pink); $\lambda_{\max}^{\mathrm{EtOH}}$ 238 (3.91), 270 (4.25), 325 (4.26), 362_{sh} (4.20), $\lambda_{\max}^{\mathrm{EtOH+AlCl_3}}$ 251 (4.33), 278 (4.38), 350 (4.25), 415 (4.26), $\lambda_{\max}^{\mathrm{EtOH+NaOAc}}$ 271 (4.39), 328 (4.15), 368 (4.20), $\lambda_{\max}^{\mathrm{EtOH+NaOEt}}$ 212 (4.26), 234 (4.13), 281 (4.00), 347 (3.71), 413 (3.95); $\nu_{\max}^{\mathrm{Nujol}}$ 3350, 3300 (OH), 1640 (CO), 1615, 1590, 1548, 1490, 1480, 1387, 1318, 1295, 1260, 1215, 1200, 1170, 1155, 1098, 1073, 1065, 1033, 1020, 970, 883, 805, 789, 762, 735, 695, 680, 585 cm^{-1}; δ (CD_3COCD_3)=3.89 (s, 3H, C_6–OMe), 6.68 (s, 1H, C_8–H), 9.25 (s, C_3 or C_7–OH), 7.60 and 8.20 (m, 5H, B-ring protons), 12.23 ppm (s, 1H, C_5–OH); *m/e* 300 (M^+), 285 (M-15, 50%), 283 (M–OH), 282 (M-H_2O), 271, 257 (M–MeCO, base), 131, 105, 77, 69, 51, 39;

Found: C, 64.88; H, 4.29%. Calcd for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03%.

The removal of the solvent from the mother liquor afforded a brown solid, which was then chromatographed on a silica-gel column using a mixture of ether and methanol, thus dividing it into four eluates. The first eluate was further chromatographed on a silica-gel column using chloroform to give pinostrobin (**18**) (0.87 g) and alnetin (**3**) (0.48 g) as yellow needles; mp 100—101°C (from *n*-hexane); $FeCl_3$ (dark green); $\lambda_{\max}^{\mathrm{EtOH}}$ 207 (4.52), 282 (4.56), 320 (3.84), $\lambda_{\max}^{\mathrm{EtOH+AlCl_3}}$ 206 (4.35), 218 (4.29), 300 (4.33), 336 (3.88), 415 (3.14), $\lambda_{\max}^{\mathrm{EtOH+NaOEt}}$ 216 (4.20), 229 (4.04), 286 (4.37), 410 (3.46); $\nu_{\max}^{\mathrm{Nujol}}$ 3350 (OH), 1660 (CO), 760, 675 cm^{-1} (monosubst. benzene-ring); δ ($CDCl_3$)=3.95, 3.97, 4.10 (s, 9H, 3 OMe), 6.69 (s, 1H, C_3–H), 7.56 and 7.95 (m, 5H, B-ring protons), 12.45 ppm (s, 1H, C_5–OH), δ (benzene)=3.83, 3.64, and 3.80 ppm (s, 3 OMe); *m/e* 328 (M^+), 314, 315 (M-15), 183, 77, 69 (base).

Found: C, 65.85; H, 4.88%. Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91%.

The second eluate gave alnustin (**3**) (0.65 g); mp 175—176°C (from *n*-hexane); $FeCl_3$ (dark green); $\lambda_{\max}^{\mathrm{EtOH}}$ 207 (4.28), 213 (4.28), 245 (4.00), 271 (4.30), 317 (4.09), $\lambda_{\max}^{\mathrm{EtOH+AlCl_3}}$ 207 (4.28), 224 (4.15), 253 (3.85), 286 (4.28), 337 (4.08),

---

[20] H. Erdtman, *Chem. Abstr.*, **40**, 1309 (1946).

[21] V. B. Mahesh and T. R. Seshadri, *J. Sci. Instr. Res.*, **13B**, 835 (1954).

392 (4.01), $\lambda_{max}^{EtOH+NaOEt}$ 215 (4.59), 226 (4.60), 289 (4.54), 385 (3.85); ν_{max}^{Nujol} 1660 (CO), 1590, 1300, 1235, 1215, 1175, 1120, 980, 800, 765, and 695 cm^{-1} (monosubst. benzene-ring); δ (CDCl$_3$)=3.89, 3.94, 3.97 (s, 9H, 3 OMe), 6.54 (s, 1H, C$_8$–H), 7.56 and 8.10 ppm (m, 5H, B-ring protons), 12.85 ppm (s, 1H, C$_5$–OH), δ (benzene)=3.23, 3.67, and 3.87 ppm (s, 3 OMe); *m/e* 328 (M$^+$, base), 327, 313 (M-15), 309, 285, 153, 118, 105, 89, 77, 69.

Found: C, 65.90; H, 4.86%. Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91%.

The third eluate was further subjected to column chromatography on silica-gel using a mixture of chloroform and methanol; this gave pinosylvin monomethyl ether (**25**) (0.25 g) and izalpinin (**5**) (70 mg).

Pinosylvin monomethyl ether (**25**); mp 119—120°C, lit,[21] 118—120°C; λ_{max}^{EtOH} 303 (4.26); ν_{max}^{Nujol} 3338 (OH), 955 (*trans*-CH=CH), 680 cm^{-1}; *m/e* 226 (M$^+$, base), 225, 211 (M-15), 210, 195 (M–OMe), 194, 165 ($C_{13}H_9^+$: Found 165.0696; Calcd. 165.0704).

Izalpinin (**5**): mp 190—191°C, lit,[21] 192—193°C; δ (CD$_3$-COCD$_3$)=3.83 (s, 3H, OMe), 6.38 (d, J=2 Hz, 1H), 6.50 (d, J=2 Hz, 1H), 7.62 and 8.17 (m, 5H, B-ring protons), 12.64 ppm (s, 1H, C$_5$–OH).

The fourth eluate was repeatedly subjected to preparative thin-layer chromatography, thus isolating tectochrysin (**6**), (25 mg), strobopinin (**19**) (12 mg), naringenin (**20**) (22 mg), alpinetin (**21**) (19 mg), and pinosylvin (**24**) (48 mg).

Tectochrysin (**6**): mp 165—166°C, lit,[21] 163—164°C; ν_{max}^{Nujol} 1660 (CO), 795, 759, 681, 632 cm^{-1}; *m/e* 268 (M$^+$, base), 255, 240, 166, 138, 123, 95, 77, 39.

Strobopinin (**19**): mp 225—226°C, lit,[20] 225—227°C; λ_{max}^{EtOH} 295 (4.33), 338 (3.70); ν_{max}^{Nujol} 1640 (CO), 810, 700 cm^{-1}; *m/e* 270 (M$^+$), 269, 193, 166 (base), 138, 120, 104, 69, 51, 39.

Naringenin (**20**): mp 251—252°C, lit,[22] 248°C; λ_{max}^{EtOH} 290 (4.33); ν_{max}^{Nujol} 3300—3110 (OH), 1634 (CO), 820 cm^{-1}; *m/e* 272 (M$^+$), 271, 179, 166, 153 (base), 152, 124, 120, 107, 91, 69, 31.

Pinosylvin (**24**): mp 156—158°C, lit,[21] 153—155°C; λ_{max}^{EtOH} 305 (4.49); ν_{max}^{Nujol} 953 (*trans* CH=CH), 675 cm^{-1}; *m/e* 212 (M$^+$, base), 211, 197 (M-15), 195, 194, 165 ($C_{13}H_9^+$: Found 165.0709; Calcd. 165.0704), 141, 128, 77, 69, 55, 51, 39.

Methylation with diazomethane yielded pinosylvin dimethyl ether (**26**).

Pinosylvin Dimethyl Ether (26). A sample of a neutral fraction (50 g) was chromatographed on a silica-gel column using a mixture of *n*-hexane and ether (1:1) to give pinosylvin dimethyl ether (**26**) as a yellow oil (0.225 g); λ_{max}^{EtOH} 305 (4.39); $\nu_{max}^{liq.}$ 950 (*trans* CH=CH), 740, 682 cm^{-1}; *m/e* 240 (M$^+$, base), 239, 225 (M-15), 209 (M–OMe), 194, 165 ($C_{13}H_9^+$: Found 165.0706; Calcd. 165.0704), 152, 77, 55. The demethylation of **26** with pyridine hydrochloride gave pinosylvin (**24**).

Methylation of Alnusin (1). *A) With Diazomethane*: The treatment of a methanol solution of alnusin (0.1 g) with excess diazomethane at 0°C for 3 hr gave 5-hydroxy-3,6,7-trimethoxy flavone (**8**) (74 mg), mp 175—176°C, which was identical with alnustin (**3**) in all respects.

B) With Dimethyl Sulfate: The refluxing of an acetone solution of alnusin (0.4 g) with dimethyl sulfate (2 m*l*) in the presence of dry potassium carbonate (3.0 g) gave 3,5,6,7-tetramethoxyflavone (**9**) (0.3 g); mp 112.0—112.5°C, lit,[7] 110—111°C; λ_{max}^{EtOH} 213 (4.50), 240 (4.21), 260 (4.30), 310 (4.30); ν_{max}^{Nujol} 1637 (CO), 1627, 1232, 973, 813, 790, 778, 701 cm^{-1}; δ (CDCl$_3$)=3.88, 3.92, 3.97, 4.02 (s, 12H, 4 OMe), 6.78 (s, 1H, C$_8$–H), 7.55 and 8.22 (m, 5H, B-ring protons), δ (benzene)=3.28, 3.75, 3.80, 4.04 (s, 3 OMe); *m/e* 342 (M$^+$), 327 (M-15, base), 323, 311, 283, 195, 167, 141, 105, 77, 69.

Found: C, 66.11; H, 5.41%. Calcd for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30%.

Acetylation of Alnusin (1). The acetylation of **1** (76 mg) with acetic anhydride in the presence of pyridine gave the triacetate (**7**) (66 mg); mp 142—144°C (from *n*-hexane-ethyl acetate); λ_{max}^{EtOH} 257 (3.89), 300 (3.80); ν_{max}^{Nujol} 1770 (acetate), 1644 (CO), 1182, 699 cm^{-1}; δ (CDCl$_3$)=2.29 (s, 3H, MeCO), 2.38 (s, 3H, MeCO), 2.45 (s, 3H, C$_5$–MeCO), 3.86 (s, 3H, C$_6$–OMe), 7.27 (s, 1H, C$_8$–H), 7.55 and 7.79 ppm (m, 5H, B-ring protons), δ (benzene)=3.69 (s, OMe); *m/e* 426 (M$^+$), 384, 342, 300 (base,) 285, 282, 271, 257, 105, 77, 69, 43.

Ethylation of Alnusin (1). The heating of the acetone solution of **1** (68 mg) with ethyl iodide (4 g) and dry potassium carbonate (2 g) for 70 hr gave the triethyl ether derivative (**10**) (40 mg); λ_{max}^{EtOH} 262 (3.69), 312 (3.64); δ (CDCl$_3$)= 1.28 (t, J=7 Hz, 3H, Me), 1.51 (t, J=7 Hz, 6H, 2 Me), 3.90 (s, 3H, OMe), 4.18 (q, J=7 Hz, 6H, 3 CH$_2$), 6.71 (s, 1H, C$_8$–H), 7.48 and 8.08 ppm (m, 5H, B-ring protons), δ (benzene)=1.09 (t, J=7 Hz, 3H, Me), 1.20 (t, J=7 Hz, 3H, Me), 1.56 (t, J=7 Hz, 3H, Me), 3.48 (q, J=7 Hz, 2H, CH$_2$), 3.48 (q, J=7 Hz, 2H, CH$_2$), 3.75 (s, OMe), 4.25 (q, J=7 Hz, 2H, CH$_2$), 4.28 (q, J=7 Hz, 2H, CH$_2$).

Methylation of Alnetin (2). The methylation of **2** (0.35 g) with dimethyl sulfate in the same manner as above yielded **11** (0.32 g); 112—113°C, lit,[9] 112—113°C; λ_{max}^{EtOH} 207 (4.40), 270 (4.34), 305 (4.08); ν_{max}^{Nujol} 1660 (CO), 763, 680 cm^{-1}; δ (CDCl$_3$)=3.96 (s, 6H, 2 OMe), 4.04 (s, 3H, OMe), 4.10 (s, 3H, OMe), 6.70 (s, 1H, C$_3$–H), 7.57 and 7.96 ppm (m, 5H, B-ring protons), δ (benzene)=3.65, 3.73, 3.77, 4.01 ppm (s, 4 OMe); *m/e* 342 (M$^+$), 327 (M-15, base), 284, 197, 182, 83.

Found: C, 66.27; H, 5.30%. Calcd for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30%.

Methylation of Alnustinol (15). The methylation of alnustinol (18 mg) with diazomethane yielded **22** (15 mg); mp 189.0—189.5°C (from benzene); λ_{max}^{EtOH} 216 (4.28), 234 (4.21), 292 (4.31), 340 (3.52), $\lambda_{max}^{EtOH+AlCl_3}$ 227 (4.29), 316 (4.36), 390 (3.22), $\lambda_{max}^{EtOH+NaOEt}$ 244 (4.42), 295 (4.35), 377 (3.87); ν_{max}^{Nujol} 3498 (OH), 1635 (CO), 765 and 698 cm^{-1}; δ (CDCl$_3$)=3.48 (bs, 1H, C$_3$–OH), 3.83 (s, 3H, C$_6$–OMe), 6.12 (s, C$_8$–H), 4.54 (d, J=12 Hz, 1H, C$_3$–H), 5.10 (d, J=12 Hz, 1H, C$_2$–H), 7.49 (s, 5H, B-ring protons), 11.03 ppm (s, 1H, C$_5$–OH); *m/e* 316 (M$^+$), 287 (M–CHO), 198, 197, (base), 196, 182, 181, 170, 167, 155, 154, 136, 125, 120, 91, 77, 69, 65, 51, 39.

Found: C, 63.95; H, 4.87. Calcd for $C_{17}H_{16}O_6$: C, 63.57; H, 4.67%.

Alkaline Degradation of 3,5,6,7-Tetramethoxyflavone (9). The flavone (**9**) (0.18 g) was refluxed in a mixture of a 50% potassium hydroxide solution (16 m*l*) and ethanol (7 m*l*) in a current of nitrogen for 20 hr. After acidification, the ether extract was washed with a 5% sodium bicarbonate solution. The ethereal layer provided 2-hydroxy-ω-4,5,6-tetramethoxy-acetophenone (**12**) (68 mg); mp 69—71°C; λ_{max}^{EtOH} 211 (4.14). 228 (4.29), 298 (4.33), 308 (4.37); ν_{max}^{Nujol} 3370 (OH), 1625 (CO), 780 cm^{-1}; δ (CDCl$_3$)=3.50 (s, 3H, ω-OMe), 3.73, 3.85, 3.99 (s, 9H, 3 OMe), 4.61 (s, 2H, $-CO\underline{H}_2-O-$), 6.23 (s, 1H, C$_3$–H), 13.12 (s, 1H, C$_2$–OH). On the other hand, the sodium bicarbonate washings gave benzoic acid (**14**); mp 121—122°C.

Alkaline Degradation of 5,6,7,8-Tetramethoxyflavone (11). The alkaline degradation of the flavone (**11**) (41 mg) for 20 hr in the same manner as above gave 2-hydroxy-3,4,5,6-tetramethoxy acetophenone (**13**); λ_{max}^{EtOH} 280 (3.74), 345 (3.16);

---

22) J. Shinoda and S. Kamieda, *Yakugaku Zasshi*, **49**, 575 (1929).

$\nu_{max}^{liq.}$ 3400 (OH), 1620 (CO), 730 cm^{-1}; δ ($CDCl_3$)=2.66 (MeCO), 3.80, 3.85, 3.85, 4.07 ppm (s, 12H, 4 OMe); the 2,4-Dinitrophenylhydrazone: mp 169—171°C, lit,[9] 168—170°C. Benzoic acid was obtained from the sodium bicarbonate washings.

*Dehydrogenation of Alnustinol (**15**).* The heating of alnustinol (40 mg) with palladium charcoal (10 mg) in the presence of cinnamic acid (0.18 g) gave 3,5,7-trihydroxy-6-methoxyflavone, which was identical to alnusin (**1**) in all respects.

The author wishes to express his hearty gratitude to Dr. Takayuki Suga and Professor Emeritus Tamon Matsuura of Hiroshima University for their guidance and encouragement. The author is also indebted to Professor Hyoji Suzuki of Hiroshima University for his suggestions about identifing *Alnus sieboldiana*, to Professor Shô Itô of Tohoku University for the measurement of the mass spectra, and to Dr. M. E. Wall of the Research Triangle Institute for the measurement of the high-resolution mass spectra. Thanks are also due to Professor H. Erdtman of the Royal Institute Technology for kindly supplying tectochrysin, pinocembrin, chrysin, and pinosylvin monomethyl ether and also for his helpful advice, and to Dr. H. H. Lee of the University of Malaya for his kind gift of the 5,6,7,8-tetramethoxyflavone used in this investigation.