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Phenolic Compounds from the Wood of Keyamahannoki

Alnus hirsuta TURCZ. (Betulaceae)^{*1,*2}

By

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ケヤマハンノキ材のフェノール性物質

笹谷宜志^{*3} 泉山紘一^{*3,*4}

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Introduction

It is well known that the wood of the alder discolors quickly to reddish brown or orange-brown when the tree is felled. The red staining substance in red alder *Alnus rubra* BONG. has long been a source of trouble in the production of lumber in the Pacific Northwest region of the United States of America. The wood of *Alnus hirsuta* TURCZ. (Betulaceae, Japanese name "Keyamahannoki") is light colored when freshly cut, but rapidly turns brown or reddish brown. On this phenomenon, Kurth, E. F. has pointed out that the red coloring matter was mainly phenolic xyloside in the wood¹⁾.

A study of the relevant literature has revealed that previous work has not been done on the constituents of the wood of Keyamahannoki *Alnus hirsuta* TURCZ., but that the extractives from the bark of grey alder *A. incana* L., black

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alder *A. glutinosa* L., green alder *A. viridis* and red alder *A. rubra* BONG. have been investigated¹⁾. Recently, ASAKAWA, M. has obtained yashabushiketol, dihydroyashabushiketol and β -phenylethyl cinnamate from the buds of *A. firma* SIEB. et ZUCC.²⁾, and diarylheptanoid 1,7-diphenylheptane-3,5-diol was isolated by UVAROVA, N. I. et al. from the leaves of *A. fruticosa* and *A. manshurica*³⁾. In the progress of our study, TERAZAWA, M. et al. reported that a compound with diarylheptanoid structure was isolated from the inner bark of Keyamahannoki *A. hirsuta*, and they proposed the name hirsutanonol, 1,7-di-(3,4-dihydroxyphenyl)-3-one-5-ol⁴⁾.

The purpose of this investigation is to determine the chemical nature and its structure of phenolic compounds in the wood of Keyamahannoki *A. hirsuta*. As the result, nine crystalline substances and vanillic acid were isolated from the ether soluble fraction of alcoholic extracts from the wood. In addition, syringic acid was confirmed in thin layer chromatography. Since the chemical nature of three of nine compounds (the named tentatively E-1 to E-9) is yet unknown in the literature, we proposed the name hannoki ester, hannokinol and hannokinin for compounds E-2, E-3 and E-4. A chemical feature of the other three compounds (E-1, E-7 and E-8) was similar to that of asadanin homologous, which was isolated by YASUE, M. from the wood of Asada *Ostrya japonica*⁵⁾, and had the structure of m, m'-bridged biphenyl. The above compound (E-2) was a new ester as we have stated in a preliminary report⁶⁾.

In this paper, three substances E-1, E-3, E-4 and further work on E-2 have now been referred to its chemical structure, and the remaining compounds will be described in a later paper.

Results and Discussion

Ten kilograms of air-dried wood meal were percolated with 95% ethanol and the extract yielded about 3.6% on oven-dried wood. An ethanolic extract was successively extracted by light petroleum ether, ethyl ether and ethyl acetate, and the fractions obtained were ca. 0.4%, 0.6% and 1.5% on oven-dried wood, respectively. Using a solution of saturated sodium bicarbonate, 10% sodium carbonate and 5% potassium hydroxide, the ether soluble portion was divided into acidic fractions and a neutral fraction. The acidic fractions obtained were 32.0 g, 22.6 g and 6.8 g from sixty five grams of ether soluble fraction, and was 3.0 g for the neutral fraction.

Nine compounds (E-1 to E-9) and vanillic acid were isolated from the acidic fraction of the ether soluble portion. The substances of the E-series are positive to diazotized reagents, e.g. of sulfanillic acid and benzidine, and ferric chloride. In the ultra violet absorption spectrum, the B-band of these compounds in a neutral medium is shifted to the long wave region on the addition of an alkali. All of these substances display the nature of phenols from the results of color reactions and spectral features.

Considering the data of UV, IR and MS spectra, the nine compounds of the E-series may be collected roughly into three groups. Group I, which contains

E-3, E-4 and E-9, have commonly the UV absorption maximum at 280 m μ area due to benzenoid nuclei, indicate the IR absorption band at 840~800 cm⁻¹ attributed to a 1,4-substituted aromatic ring. On degradation with potassium permanganate, the methylated substances of E-3 and E-4 yield *p*-anisic acid. The MS spectrum of the above compound reveals the presence of a prominent ion peak at m/e 107 (base ion peak) due to ((HO)Ar-CH₂)⁺. It is suggested that these compounds contain *p*-hydroxyphenyl nuclei as the partial structure.

Group II consists of E-1, E-5, E-7 and E-8. They have a UV absorption maximum at 300~310 m μ . Their behaviour in alkaline medium and its differential curve are similar to that of asadanin and its related compounds from the wood of Asada *Ostrya japonica*. On degradation with potassium permanganate, the methylated materials of group II gave a reactant with biphenyl nuclei, namely 2,2'-dimethoxy-5,5'-dicarboxybiphenyl. The MS spectra of E-1 and E-7 indicate a prominent ion peak at m/e 211 originated in biphenyl nuclei. Therefore, compounds belonging to group II appear to contain biphenyl moiety in the structure. This suggestion also supports their solubility in alkali solutions. When the ether soluble fraction was treated with alkali, E-3 and E-4 are mainly fractionated by 5% potassium hydroxide, while compounds of Group II were dissolved in 10% sodium carbonate. The acidity of E-1, therefore, is higher than that of E-3. YASUE, M. has pointed out that the pK values of asadanin (XI) were pK₁ 8.9 and pK₂ 13.0, and pK₁ was considerably lower than the common phenols (e.g. phenol pK 10.00; *p*-cresol pK 10.17)⁵⁾. It appears to be proof that one of two hydroxyl groups on biphenyl nuclei of asadanin was firmly linked to the oxygen atom of another hydroxyl group through the hydrogen bond, and in consequence a hydrogen atom, which was not involved in the hydrogen bond, was easily subjected to dissociation.

Group III contains the remaining materials E-2 and E-6.

Trideoxysadanin-8-ene (E-1) (I)

Compound I, one of group II, mp 238~241°C, C₁₉H₁₈O₃ (m/e 294 M⁺), is positive to diazo-reagents and ferric chloride, but negative to quinone mono-chlorimide and magnesium-hydrochloric acid test. It gave a diacetate (Ia) with acetic anhydride and pyridine, and dimethyl ether (Ib) by dimethyl sulphate. The UV spectrum of compound I has absorption maxima at 216 and 297 m μ , and the latter maximum shifts toward 327 m μ adding alkali. Its behaviour in alkaline medium and its differential curve is very similar to that of asadanin and its related compounds. The IR spectrum of E-1 shows absorption maxima at 3,270 cm⁻¹ originated in phenolic hydroxyl group, 1,675 cm⁻¹ due to α , β -unsaturated carbonyl group, 1,615 cm⁻¹ attributed to an olefinic double bond, 1,600 and 1,500 cm⁻¹ derived from phenyl group. This suggested the presence of an α , β -unsaturated ketone which did not conjugated to hydroxyphenyl nuclei (Figs. 1 and 2).

On the oxidation of methyl ether (Ib) with potassium permanganate, compound Ib gave a 2,2'-dimethoxy-5,5'-dicarboxybiphenyl, which was identified with an authentic specimen on TLC.

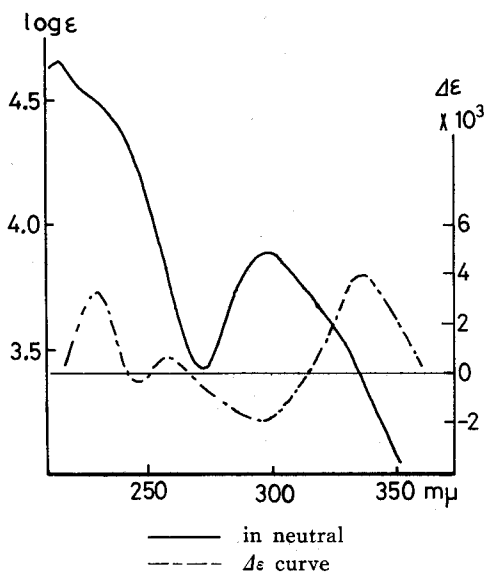


Fig. 1. Ultra violet absorption spectrum and its $\Delta\epsilon$ curve of E-1 (trideoxysadanin-8-ene).

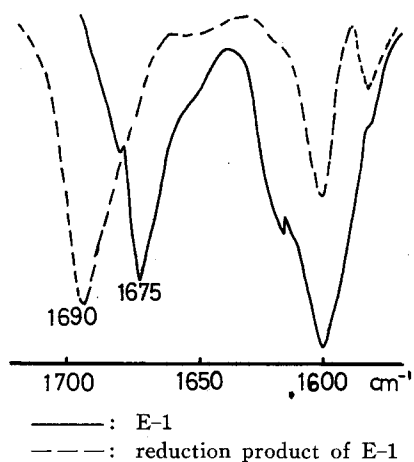


Fig. 2. Carbonyl bands in Infra red absorption spectra of E-1 and reduction product.

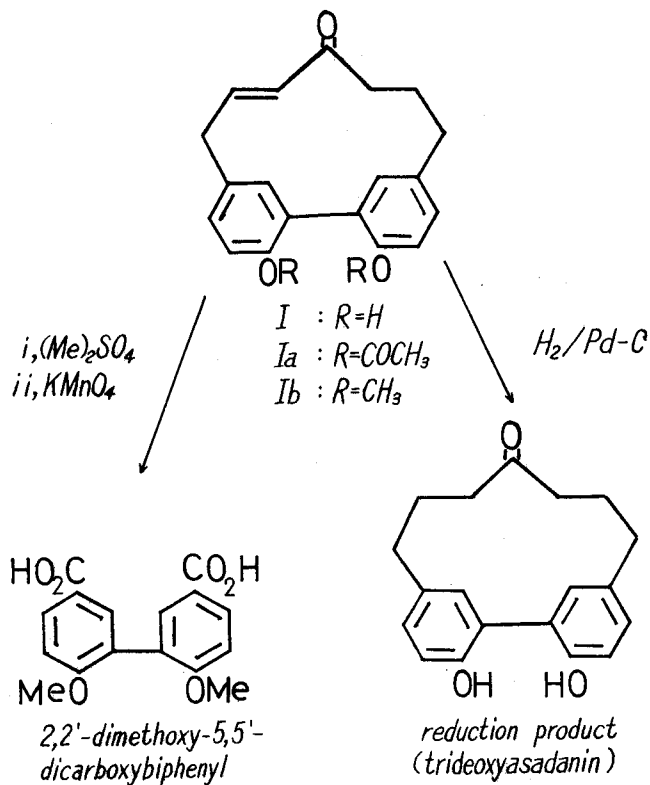


Fig. 3. Degradation and reduction products from E-1.

From these results, one of the three oxygen atoms in the structure must be placed in side chain and the remaining oxygen atoms consisted of two hydroxyl groups on biphenyl moiety. Also compound I absorbs a mole of hydrogen by catalytic reduction with Pd-C. This supports from the result of IR spectrum of the reactant, namely an absorption maximum at $1,615\text{ cm}^{-1}$ (E-I) disappeared and a carbonyl band at $1,675\text{ cm}^{-1}$ shifted at $1,690\text{ cm}^{-1}$. It indicates that an olefinic double bond was saturated. This reduction product was identified with trideoxy-asadanin, which was yielded by the clemmensen reduction of asadanin, on TLC and the mixed melting point.

The NMR spectrum of Ia in deuteriochloroform reveals signals at 2.90 (1H,

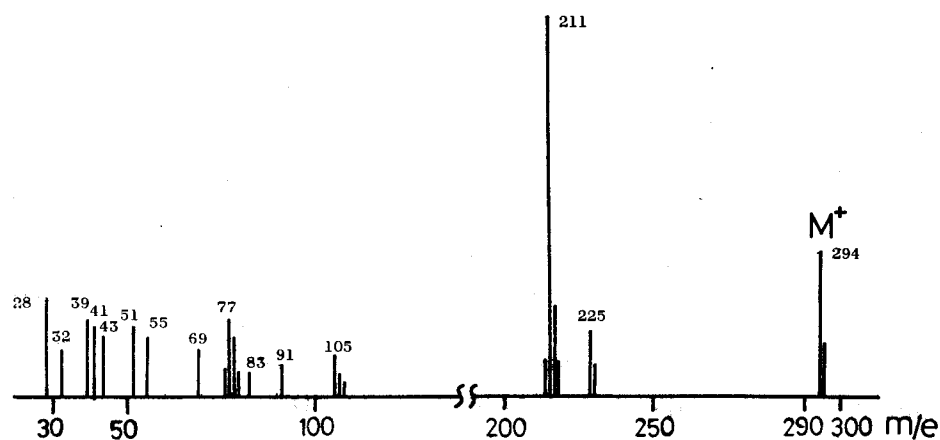


Fig. 4. Mass spectrum of E-1 (trideoxyasadanin-8-ene).

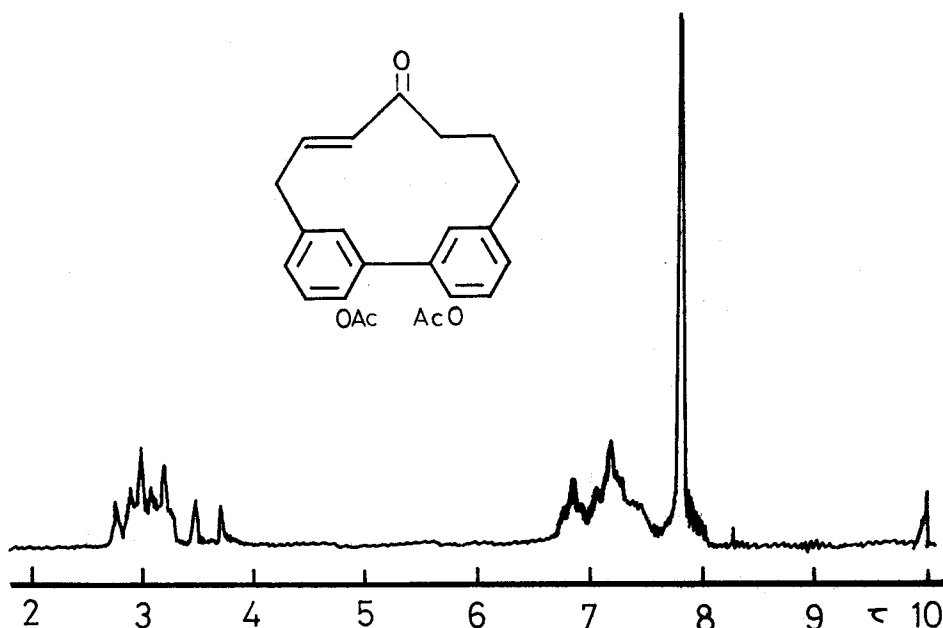
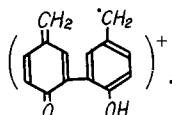


Fig. 5. NMR spectrum of E-1 diacetate.

doublet, $J=16$ Hz), 3.0 (6H, multiplet), 3.61 (1H, doublet, $J=16$ Hz), 6.6~7.7 (8H) and 7.81 (6H, singlet) τ . A pair of doublet at 2.90 and 3.61 indicates the presence of an olefinic protons, and a multiplet around 3.0 reveals six protons of biphenyl moiety. The broad signal at 6.6~7.7 is due to two methylene protons of the benzyl group and two other methylene protons in side chain. A singlet at 7.81 shows two acetoxyl protons on biphenyl nuclei (Fig. 5).

Furthermore, a structural assignment on biphenyl nuclei can be confirmed by the result that a prominent ion in the MS spectrum of compound I was observed

at m/e 211 as the base ion peak due to the fragment .

All of these data on compound I support the structure I, and E-1 is determined to be trideoxyasadanin-8-ene, which was isolated by YASUE, M. from the wood of *Asada Ostrya japonica*. Trideoxyasadanin-8-ene has a carbon skeleton of $C_6-C_7-C_6$ not encountered in other naturally occurring substances. However, several compounds having a $C_6-C_7-C_6$ skeleton have been studied during the past twelve years, e.g. asadanin in *Ostrya*, cetrolobine in *Centrolobium*⁷⁾ and yasha-bushiketol in *Alnus*, and curcumin in *Curcuma* prior to this. It is very interesting that these compounds with a $C_6-C_7-C_6$ carbon skeleton occur among the genus and between the genus in the same family, Betulaceae. It appears to be have a strong resemblance to those biosynthetic path way.

Hannoki ester (β -guaiacyl ethyl ferulate) (E-2) (II)

We proposed the name hannoki ester for this compound II (mp 194~195°C, $C_{19}H_{20}O_6$, m/e 344 M^+) in a preliminary report. All of the data of UV (Fig. 6), IR (Fig. 7), NMR (E-2 diacetate, Fig. 10) spectra, and the behaviour of hydrolytate (I and II) and of a degradation product had substantiated β -guaiacyl ethyl ferulate for the structure of compound II. The final confirmation of its structure has been achieved by the analysis of hannoki ester dimethyl ether and by a comparison with a synthetic methyl ether of β -guaiacyl ethyl ferulate. The chemical nature of methyl ether IIb is now described in this paper.

Treated with diazomethane, hannoki ester gave its dimethyl ether IIb as a colorless needles, mp 123.9°C. The NMR spectrum of compound IIb in deuteriochloroform with tetramethylsilane as an internal standard reveals the following signals: 2.37 (1H, doublet, $J=16$ Hz), 2.88~3.32 (6H, multiplet), 3.71 (1H,

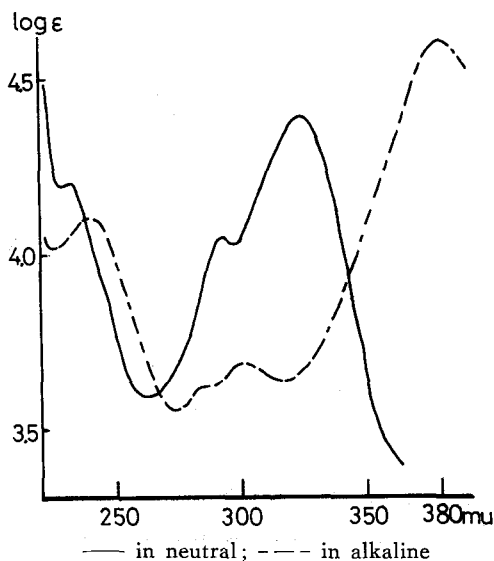


Fig. 6. Ultra violet absorption spectra of E-2 (β -guaiacyl ethyl ferulate).
— in neutral; --- in alkaline

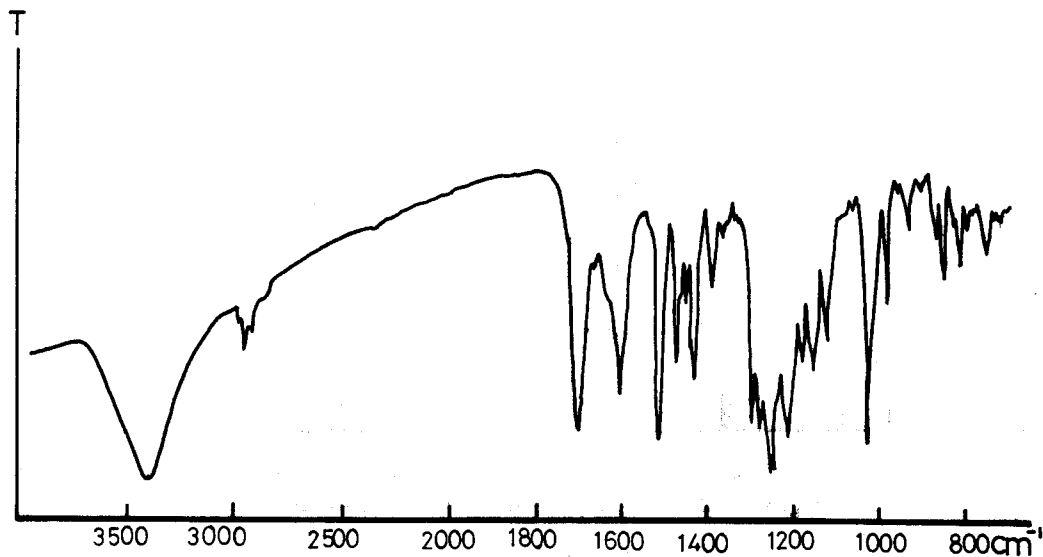


Fig. 7. Infra red absorption spectrum of E-2 (β -guaiacylethyl ferulate).

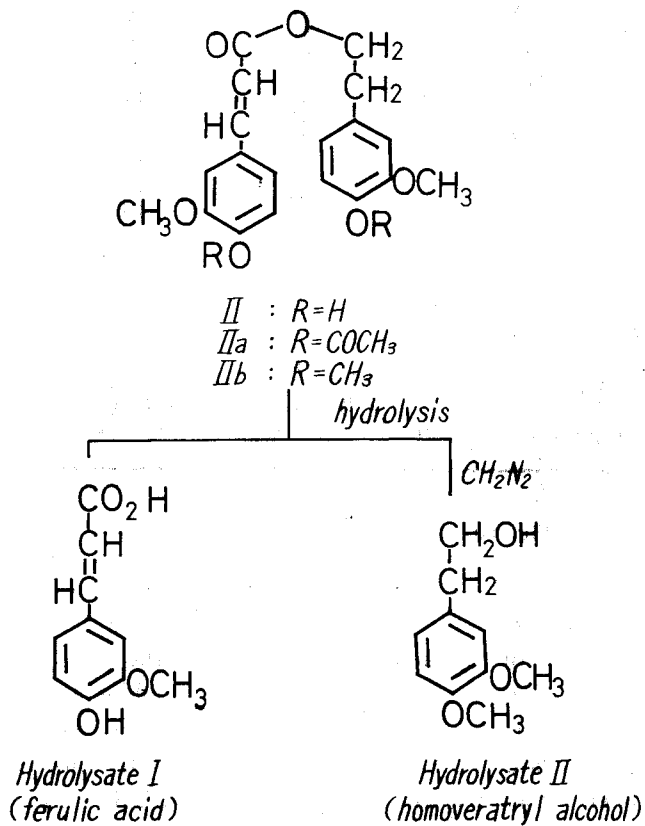


Fig. 8. Hydrolysates from E-2 with alcoholic potassium hydroxide.

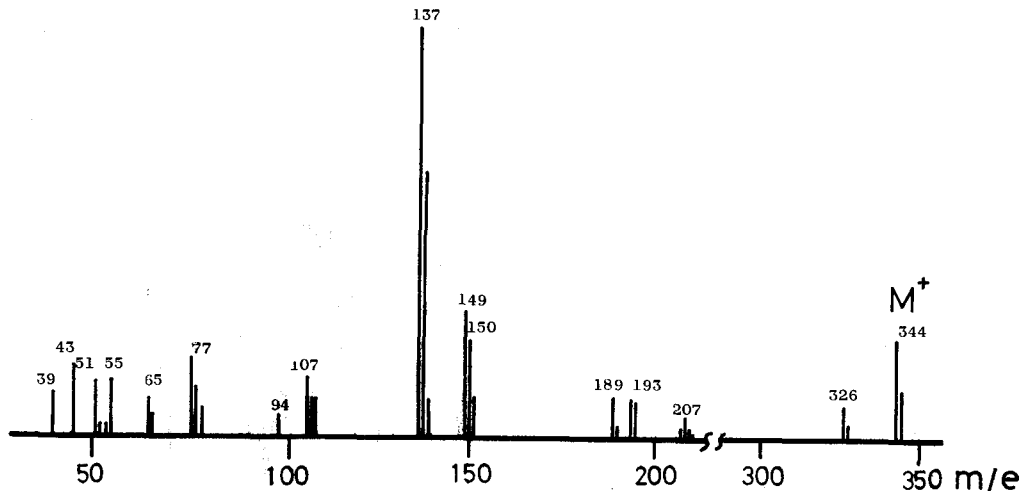


Fig. 9. Mass spectrum of E-2 (β -guaiacyl ethyl ferulate).

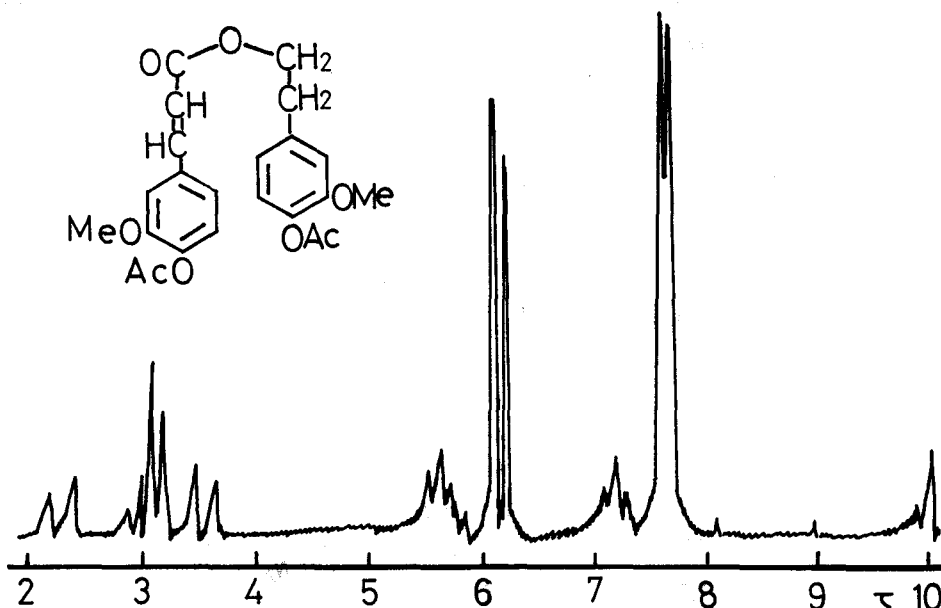


Fig. 10. NMR spectrum of E-2 diacetate.

doublet, $J=16$ Hz), 5.63 (2H, triplet, $J=7$ Hz), 6.12 (6H, singlet), 6.18 (6H, siglet), 7.12 (2H, triplet, $J=7$ Hz) τ (Fig. 11). A feature of the NMR spectrum of IIb is very similar to that of hannoki ester diacetate IIa (Fig. 10), apart a signal attributed to acetoxy protons at 7.68 τ . A pair of doublet at 2.37 ($J=16$ Hz) and 3.71 ($J=16$ Hz) indicated the presence of an olefinic double bond in 3, 4-dimethoxycinnamic acid residue. A multiplet at 2.88~3.37 showed six protons of two aromatic nuclei. Two triplets at 5.63 (C_α) and 7.21 (C_β) are due to the partial structure of $-\text{CO}-\text{O}-\text{CH}_2(\alpha)-\text{CH}_2(\beta)-\text{Ar}(\text{OCH}_3)_2$, respectively. The behaviour of protons of methylene at C(α) adjacent to the oxygen atom also agrees very well

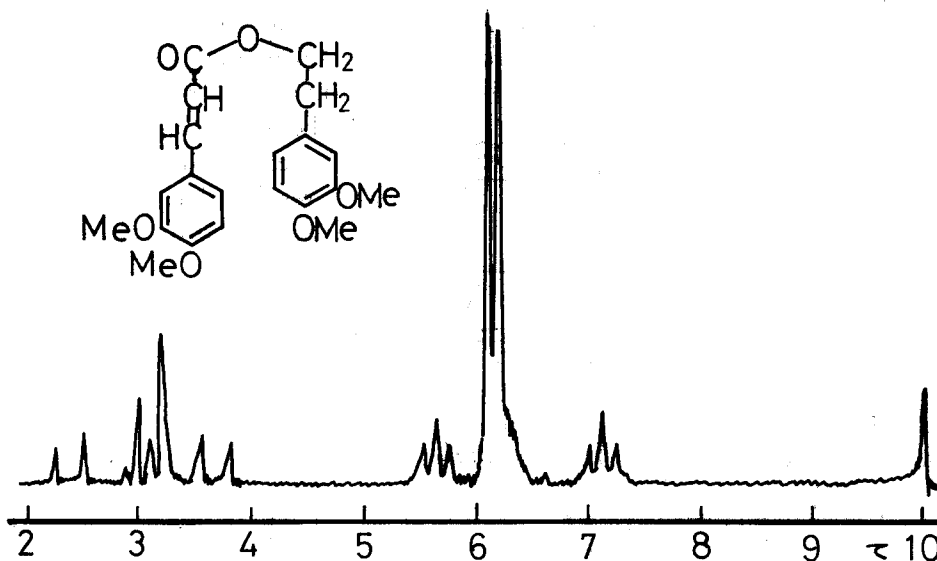


Fig. 11. NMR spectrum of E-2 dimethyl ether.

with that of guaiacylethane- β -dihydroconiferyl ether⁸⁾. Two singlets at 6.12 and 6.18 can be assigned four methoxyl protons of two aromatic nuclei.

Furthermore, the structural assignment is supported by a comparison with synthetic dimethyl ether of β -guaiacylethyl ferulate and the mixed melting point. Synthetic dimethyl ether was prepared from a homoveratryl alcohol and a 3,4-dimethoxycinnamic acid chloride. The melting point of the reactant was 124.3°C and the mixed melting point with IIb was undepressed. Also, the NMR spectrum of the synthetic compound agreed with that of substance IIb.

Finally, this ester consists of a ferulic acid and a homovanillyl alcohol and is concluded to be β -guaiacylethyl ferulate. This compound having C₆-C₃ and C₆-C₂ carbon skeleton is apparently a novel type, as is asadanin in nature, though only β -phenylethyl cinnamate occurred in the buds of Yashabushi *Alnus firma* SIEB. et ZUCC. (Betulaceae).

Hannokinol (1,7-di-(*p*-hydroxyphenyl)-heptane-3,5-diol) (E-3) (III)

E-3 was isolated from a 5% potassium hydroxide soluble portion of the ether soluble fraction using silica gel column chromatography and one of group 1. By several recrystallizations from 80% ethanol and benzene: acetone (1:1), this compound III was obtained as colorless plates, mp 165~166°C, and was optically active, $[\alpha]_D^{25} = +32.5$ ($c=0.53$ in EtOH). It gave positive coloration with diazo-reagents, ferric chloride and alkali-alkyl xanthate, and was negative to quinone monochlorimide, suggesting the presence of the alcoholic and phenolic hydroxyl groups in the structure. The molecular formula is established as C₁₉H₂₄O₄ from a high-resolution mass spectrum. The UV spectrum shows maxima at 225, 279.5 and 286 (shoulder) m μ , and in alkaline medium at 227.5, 243, 289 and 300 m μ , attributed to phenolic moiety (Fig. 13). The IR spectrum of III indicates absorp-

tion band at $3,400$ and $1,110\text{ cm}^{-1}$ due to secondary alcoholic hydroxyl group, $3,270\text{ cm}^{-1}$ attributed to phenolic hydroxyl group, $1,600$ and $1,508\text{ cm}^{-1}$ showed the presence of phenyl nuclei and $840\sim 800\text{ cm}^{-1}$ originating from the 1,4-substituted benzene ring (Fig. 14). It

may be easily considered from the above results that the hannokinol had the *p*-hydroxyphenyl group. Also, a carbon atom (α -position)

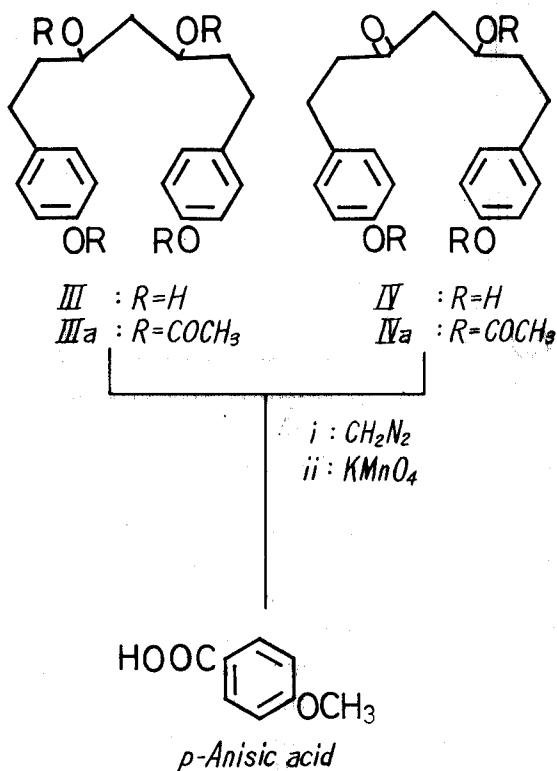


Fig. 12. Degradation product from methyl ether of E-3 and E-4 with potassium permanganate.

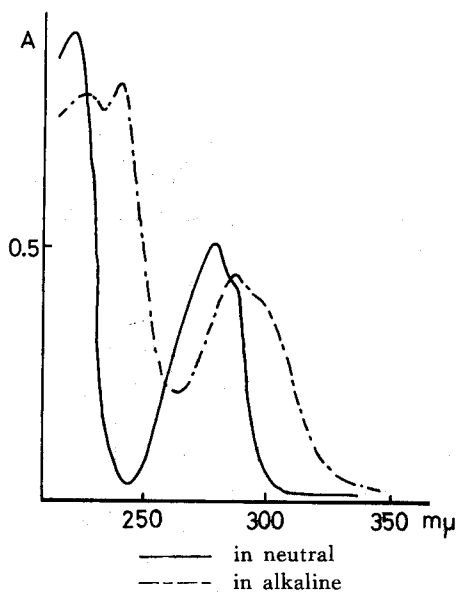


Fig. 13. Ultra violet absorption spectra of E-3 (1,7-di-*p*-hydroxyphenyl)-heptane-3,5-diol).

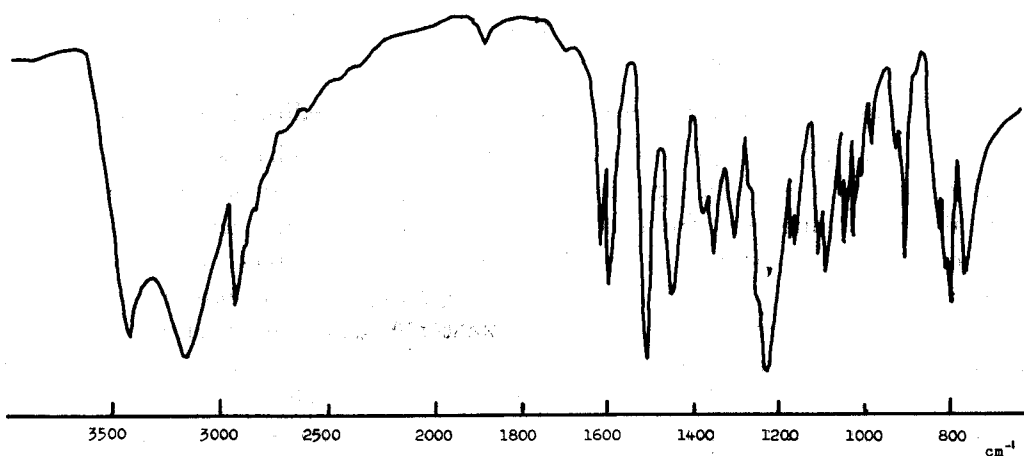


Fig. 14. Infra red absorption spectrum of E-3 (1,7-di-*p*-hydroxyphenyl)-heptane-3,5-diol).

adjacent to the benzene ring did not possess a hydroxyl group. Furthermore, this is supported by the fact that methylated hannokinol gave *p*-anisic acid by oxidation with potassium permanganate.

When hannokinol in pyridine was treated with acetic anhydride, a faint yellow, viscous tetraacetate (IIIa) was obtained. The MNR spectrum of compound IIIa shows the following signals: 2.79~3.08 (8H, multiplet), 5.02 (2H, quintet, $J=7$ Hz), 7.43 (4H, two triplets partially overlapping, $J=7$ Hz), 7.78 (6H, singlet), 8.04 (6H, singlet) and 8.19 (6H, multiplet) τ (Fig. 15). A multiplet at 2.79~3.08 indicates eight protons of two aromatic nuclei, AA' BB' system, and two singlets at 7.78 and 8.04 reveal protons of two phenolic and alcoholic acetoxy groups, respectively. A quintet at 5.02 is due to two protons of the partial structure of $-\text{CH}_2-\text{CHOAc}-\text{CH}_2-\text{CHOAc}-\text{CH}_2-$. Two of the four hydroxyl groups can be proved to be secondary alcohol from the results of coloration, IR and NMR spectra, and then the others to be phenolic hydroxyl groups. This resembles the results of NMR spectrum of yashabushiketol (VII) and its dihydro-derivatives (VIII), which were extracted from the buds of Yashabushi *Alnus firma*. The signal at 7.45 ($J=7$ Hz) shows four protons of the triplets partially overlapping two $-\text{CH}_2-\text{CH}_2-\text{Ar}(\text{OAc})$. A complex at 8.19 could be assigned to six protons of three methylene of the structure $-\text{CH}_2-\text{CHOAc}-\text{CH}_2-\text{CHOAc}-\text{CH}_2-$. The feature of three methylene groups adjacent to secondary alcohol is clearly recognized in comparison with the NMR spectrum in deuteromethanol of 1,7-diphenylheptane-3,5-diol (IX), which obtained from the leaves of *Alnus fruticosa* and *A. manshurica*.

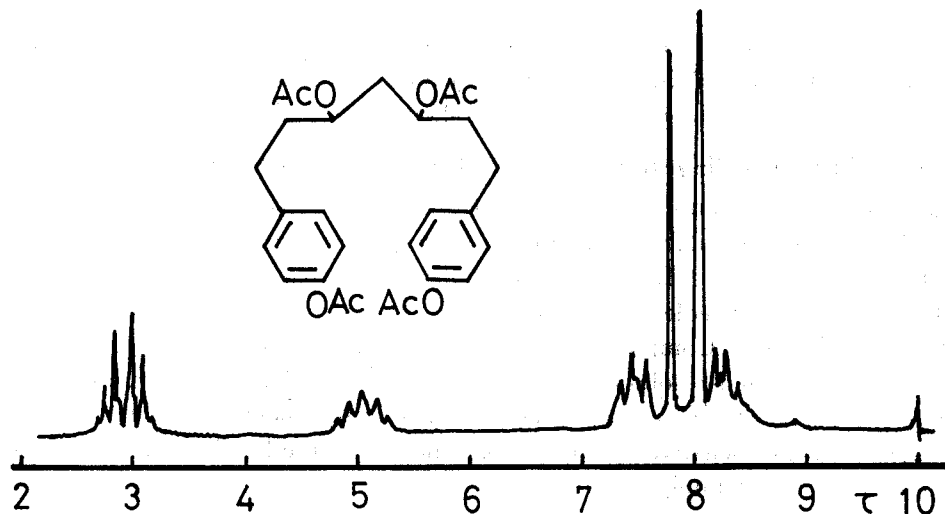


Fig. 15. NMR spectrum of E-3 (1,7-di-(*p*-hydroxyphenyl)-heptane-3,5-diol) tetraacetate.

It is not hard to appreciate from the result of MS spectrum that hannokinol contained the molecular ion peak at m/e 316, the base ion peak at 107 corresponding to $((\text{OH})\text{Ar}-\text{CH}_2\cdot)^+$, the prominent ion peaks due to M-18 (m/e 298) and

M-36 (m/e 280), of which evidence indicated to be an alcohol (Fig. 16). The characteristic fragments observed at m/e 91, 77, 65, 51 and 39 originated from substituted aromatic derivative. Therefore, it can be understood that hannokinol has two alcoholic hydroxyl groups and *p*-hydroxyphenyl nuclei in the structure. Other abundant ion peaks in the MS spectrum may be explained as follows: m/e 173 ($(OH)Ar-CH_2-CH=CH-CH=CH-CH_2$)⁺, m/e 160 ($CH_2=CH-CH=CH-CH_2-Ar(OH)$)⁺, m/e 150 ($O=CH-CH_2-CH_2-Ar(OH)$)⁺, m/e 149 ($O=C-CH_2-CH_2-Ar(OH)$)⁺, m/e 133 ($\cdot CH=CH-CH_2-Ar(OH)$)⁺, m/e 121 ($\cdot CH_2-CH_2-Ar(OH)$)⁺ and m/e 120 ($CH_2=CH-Ar(OH)$)⁺ (Fig. 16).

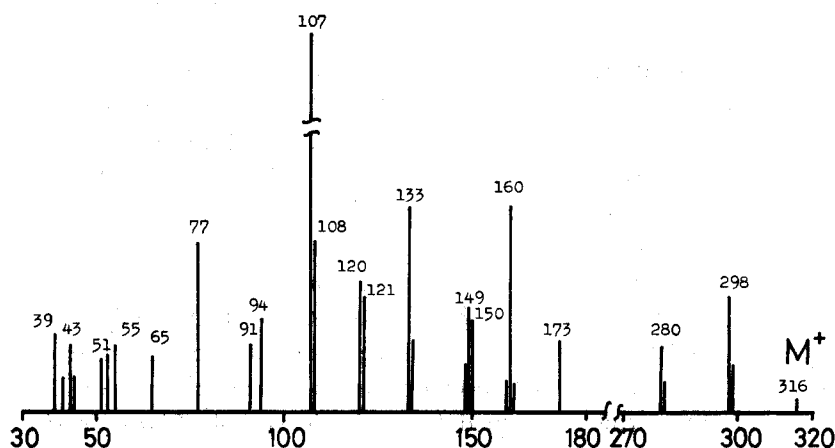


Fig. 16. Mass spectrum of E-3 (1, 7-di-(*p*-hydroxyphenyl)-heptane-3, 5-diol)

On the basis of above-stated chemical and spectral data and in comparison with the results of 1, 7-diphenyl-heptane-3, 5-diol, the structure of hannokinol is determined to be 1, 7-di-(*p*-hydroxyphenyl)-heptane-3, 5-diol.

Hannokinin (1, 7-di-(*p*-hydroxyphenyl)-heptane-3-one-5-ol) (E-4) (IV)

E-4, like hannokinol, is obtained from a 5% potassium hydroxide soluble portion of the ether soluble fraction by silica gel column chromatography and is classified into group I. By recrystallization with benzene:acetone (1:1), E-4 is yielded as colorless needles, mp 131~132°C, and is optically active, $[\alpha]_D^{25} = +20.7$ ($c=0.72$ in EtOH). Compound IV has a molecular formula of $C_{19}H_{22}O_4$ from the results of a high-resolution mass spectrum and of elemental analysis. It is positive to diazo-reagents, ferric chloride, 2, 4-dinitrophenylhydrazine and alkali-alkly xanthate, and negative to the quinone monochlorimide test. It may obviously be suggested that hannokinin contains to alcoholic and phenolic hydroxyl groups and a carbon atom (α -position) adjacent to the aromatic ring is lacking for the hydroxyl group. Also, compound IV is a ketol from the behaviour of coloration. The UV spectrum of hannokinin shows absorption maxima at 225, 280 and 285~288 (shoulder) $m\mu$, and in an alkaline medium at 246, 288 and 298 $m\mu$, suggesting phenolic moiety (Fig. 17). The manner of UV spectra in neutral and alkaline media is very similar to that of hannokinol. A significant absorption band in

the IR spectrum of E-4, however, observes at $1,690\text{ cm}^{-1}$, attributed to carbonyl group, which could not be encountered in the IR spectrum of Hannokinol (Fig. 18). Absorption bands at $3,400$ and $1,110\text{ cm}^{-1}$ indicate the presence of a secondary alcoholic hydroxyl group compared with compound III and at $3,270\text{ cm}^{-1}$ according to the phenolic hydroxyl group. Absorption maxima of phenyl nulcei are observed at $1,600$ and $1,500\text{ cm}^{-1}$, and furthermore an other absorption band at $840\sim 800\text{ cm}^{-1}$ suggests the existence of a 1,4-substituted benzene ring. It can be assumed that compound IV was closely related to the chemical structure of hannokinol, though the latter was lacking in a carbonyl group. In addition, the similarity of thier structures was detected from the result that methylated hannokinol gave *p*-anisic acid on the oxidation with potassium permanganate.

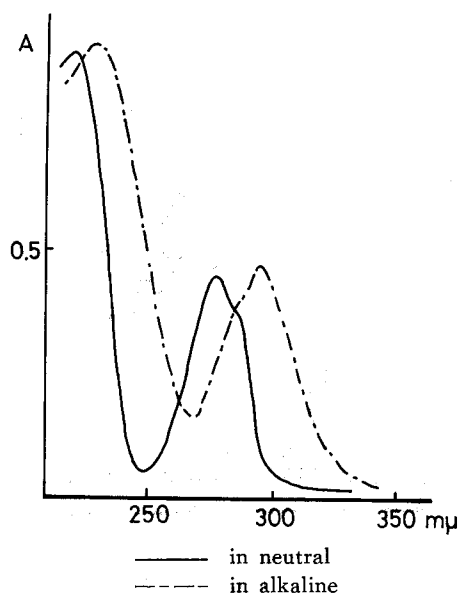


Fig. 17. Ultra violet absorption spectra of E-4 (1,7-di-*p*-hydroxyphenyl)-3-one-5-ol).

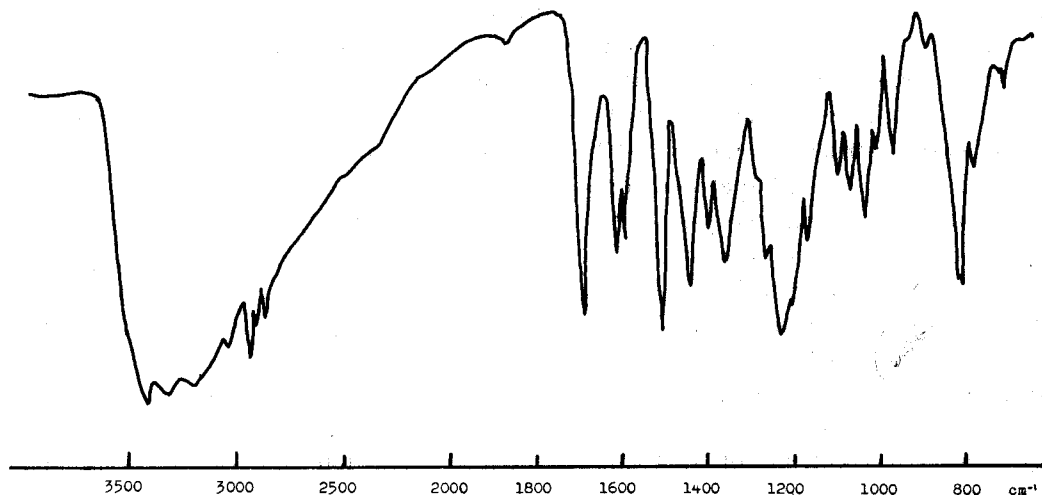


Fig. 18. Infra red absorption spectrum of E-4 (1,7-di-*p*-hydroxyphenyl)-heptane-3-one-5-ol)

The acetylation of compound IV with acetic anhydride in pyridine gave a pale yellow, viscous triacetate IVa. The NMR spectrum of IVa in deuterochloroform shows the following signals: at 2.96 (8H, multiplet), 4.75 (1H, quintet, $J=7\text{ Hz}$), 7.23~7.46 (8H, multiplet), 7.77 (6H, singlet), 8.06 (3H, singlet) and 8.16 (2H, multiplet) τ (Fig. 19). A multiplet located at 2.93 is derived from eight protons of two aromatic nuclei, AA' BB' system, and two singlets at 7.77 and 8.06

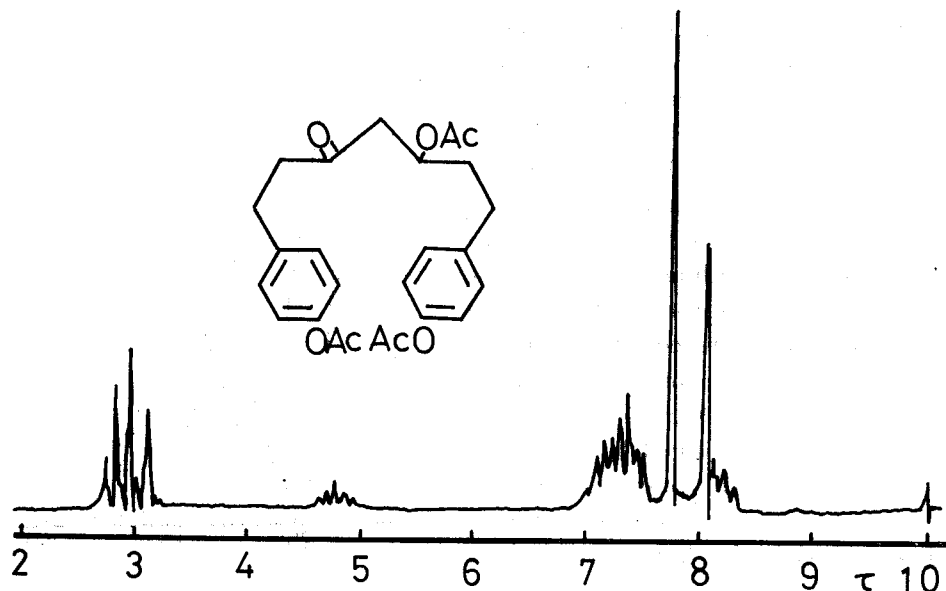


Fig. 19. MNR spectrum of E-4 (1,7-di-(*p*-hydroxyphenyl)-heptane-3-one-5-ol) triacetate.

originate in two phenolic acetoxy groups and an alcoholic acetoxy moiety, respectively. A quintet appearing in 4.75 is attributable to a proton of the partial structure $-\text{CH}_2-\text{CHOAc}-\text{CH}_2-$, and a multiplet observed at 8.16 is caused by two protons of methylene of the structure $-\text{CHOAc}-\text{CH}_2-\text{CH}_2-\text{Ar}(\text{OAc})$. A complex signal presented at 7.23~7.46 can be illustrated by overlapping four protons of methylene with two $(\text{OAc})\text{Ar}-\text{CH}_2-\text{CH}-$ groups and four protons of two methylene of the partial structure $-\text{CH}_2-\text{CO}-\text{CH}_2-$. Compared with the data of hannokinol, it has been recognized that compound IV consists of a secondary alcohol group, a carbonyl group and two *p*-hydroxyphenyl moiety as the partial structure.

In the progress of our study, TERAZAWA, M. et al. reported that platyphyl-lonol (1,7-di-(*p*-hydroxyphenyl)-heptane-3-one-5-ol), mp 125~126°C, isolated from the inner bark of Shirakanba *Betula platyphylla* and hirsutanonol, as oily state, from the inner bark of Keyamahannoki *A. hirsuta*⁴⁾. These compounds have the structure of C₆-C₇-C₆ carbon skeleton and its NMR spectra are very similar to that of hannokinol.

The MS spectrum of E-4 shows the molecular ion peak at *m/e* 314 and a prominent ion peak corresponding to M-18 at *m/e* 296 (Fig. 20). The base ion peak presented at *m/e* 107, attributing to $((\text{OH})\text{Ar}-\text{CH}_2\cdot)^+$. The presence of characteristic fragment ion peaks at *m/e* 91, 77, 65, 51 and 39 are due to a substituted aromatic ring. This also coincides with the results from hannokinol. Other mainly fragment ion peaks in the MS spectrum may be understood as follows: *m/e* 164 $((\text{OH})\text{Ar}-\text{CH}_2-\text{CH}_2-\text{CO}-\text{CH}_3)^+$, *m/e* 175 $((\text{OH})\text{Ar}-\text{CH}_2-\text{CH}_2-\text{CO}-\text{CH}=\text{CH}\cdot)^+$ or $(\cdot\text{OC}-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_2-\text{Ar}(\text{OH}))^+$ and *m/e* 190 $((\text{OH})\text{Ar}-\text{CH}_2-\text{CH}_2-\text{CO}-\text{CH}=\text{CH}-\text{CH}_3)^+$ or $(\text{CH}_3-\text{CO}-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_2-\text{Ar}(\text{OH}))^+$. These ion peaks could not found in the MS spectrum of compound III.

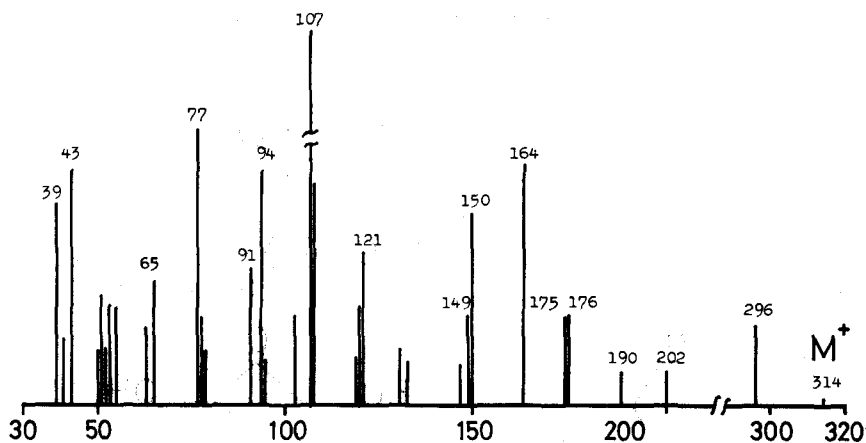
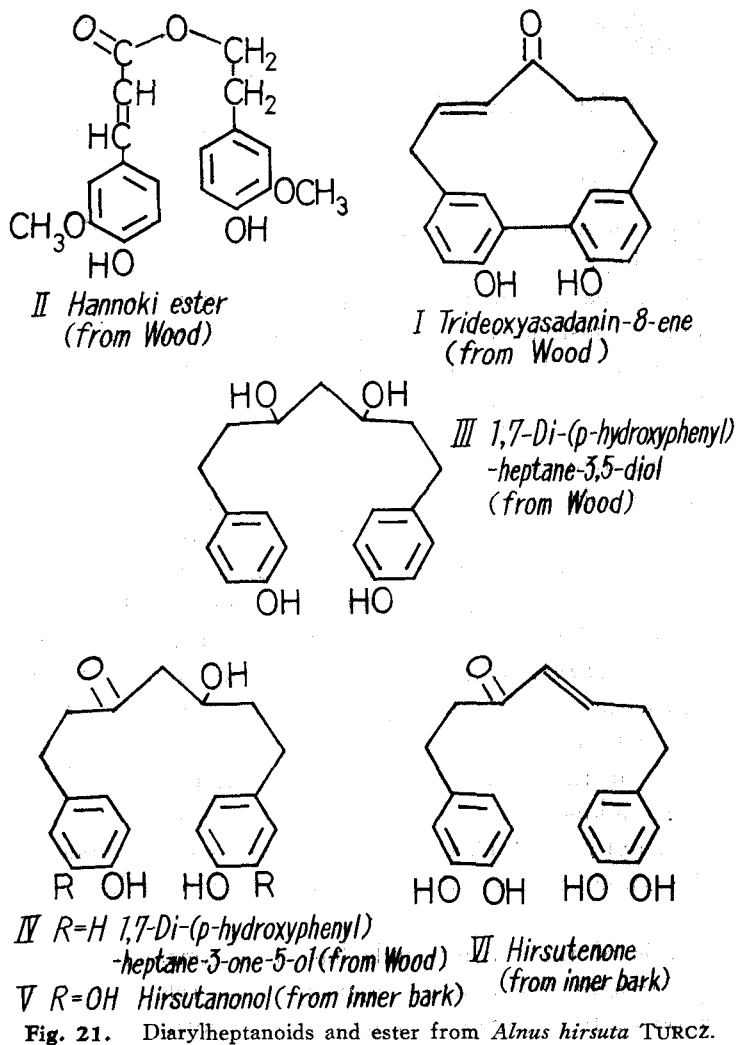
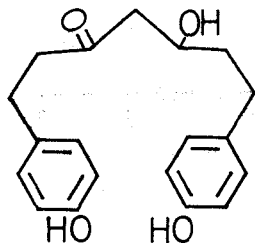
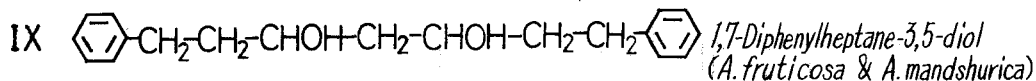
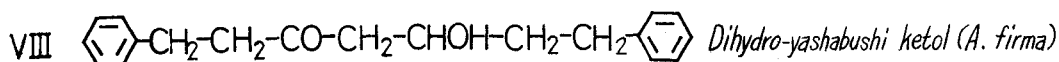
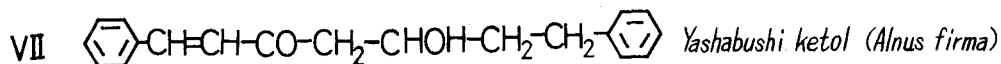
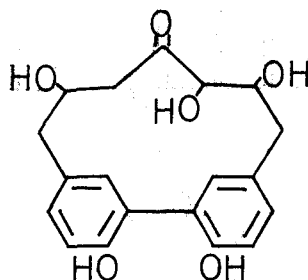


Fig. 20. Mass spectrum of E-4 (1,7-di-(*p*-hydroxyphenyl)-heptane-3-one-5-ol).





X Platyphyllonol (*Betula platyphylla*)



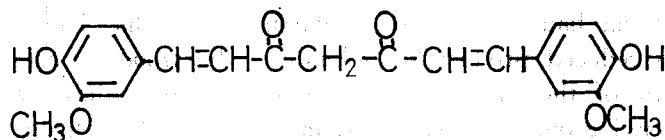
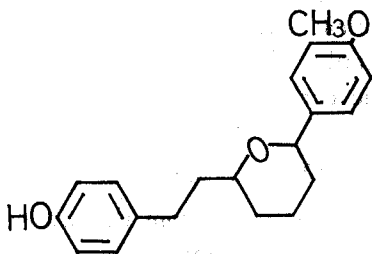
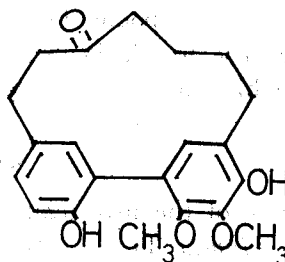
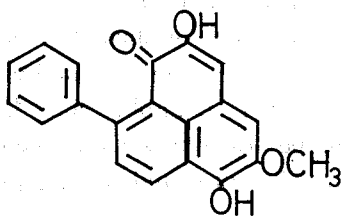
XI Asadanin (*Ostrya japonica*)

Fig. 22. Diarylheptanoids in Betulaceae.

From the above-mentioned chemical and spectral feature and comparison with data of other diarylheptanoids from *Alnus* and *Betula*, the structure of hannokinin is concluded to be 1,7-di-(*p*-hydroxyphenyl)-heptane-3-one-5-ol. Therefore, hannokinin is almost justifiably considered as coinciding with the structure of platyphyllonol, though the latter differed from hannokinin on melting point and its optical activity was as yet unknown.

Five compounds, including E-1, E-3, E-4 and two substances (E-7 and E-8) ignored in this paper, obtained from the wood of *A. hirsuta*, are all diarylheptanoids with a C₆-C₇-C₆ carbon skeleton. A few compounds related to diarylheptanoids occurred over the family in nature, e.g. curcumin (XII) in *Curcuma longa* (Zingiberaceae), centrolobine (XIII) in *Centrolobium robustum* (Legminosae), asadanin (XI) in *Ostrya japonica* (Betulaceae), myricanone (XIV) in *Myrica nagi* (Myricaceae), platyphyllonol (X) in *Betula platyphylla* (Betulaceae) and yashabushiketol (VII) in *A. firma* (Betulaceae). It is interesting concerning its biosynthesis that these compounds having a C₆-C₇-C₆ carbon skeleton occurred among the genus *Alnus*, *Betula* and *Ostrya* in the same family. Furthermore, it must be noted that diarylheptanoids hannokinol and hannokinin from the wood of *A. hirsuta* possessed *p*-hydroxyphenyl group in its structure, whereas hirsutanonol (V) from the inner bark of the same species contained the *o*-dihydroxyphenyl pendant group (Figs. 21, 22 and 23).

The biosynthesis of these compounds has been considered as the following: Curcumin from *Curcuma longa* would appear to be related to that of lignans, involving the union of two cinnamate units with a central methylene supplied by

XII Curcumin (*Curcuma*: Zingiberaceae)XIII Centrolobine (*Centrolobium*:
Leguminosae)XIV Myricanone (*Myrica*:
Myricaceae)XV 9-Phenylperinaphthenone (*Lachnanthes*:
Haemodoraceae)Fig. 23. Diarylheptanoids and related compounds
from other families in nature.

malonate⁹). YASUE, M. has reported that asadanin and its homologous may make the cyclization into the biphenyl ring by oxidative coupling after the C₆-C₇-C₆ intermediate was formed⁹). On biosynthesis of 9-phenylperinaphthenone (XV) in *Lachnanthes tinctoria* (*Haemodoraceae*), EDWARDS, J. M. et al. have pointed out that this compound was formed by elimination of acetate carbonyl group from two cinnamate units and an acetate unit during biosynthesis¹⁰). ROUGHLEY, P. J. et al., however, have proposed the following path way by tracer experiment for curcumin occurrence. A cinnamate unit and five malonate initially formed a chain expansion intermediate, and subsequently aromatized to a C₆-C₇-C₆ skeleton. Then hydroxylation and methylation led to curcumin via the above C₆-C₇-C₆ intermediate¹¹). Compounds III and IV will occur by either path way.

When regard is paid to the fact that mono-hydroxyphenyl group during biosynthetic progress was subjected further hydroxylation toward di-substituted derivative¹²), the occurrence of hannokin and hirsutanonol appears to be alternative process in xylem and phloem to each other. Therefore, hirsutanonol cannot be

expected to form via hannokinin. It is worth noting that hannokinin occurred together with its reduced product hannokinol in the wood. Furthermore, it may be a characteristic of the constituents of *A. hirsuta* that these compounds have hydroxylated phenyl group, whereas substances from other *Alnus* consist of the phenyl group lacking the hydroxyl group. As described above, the structure of hannokinin seems to be identical with that of platyphyllonol from the inner bark of *Betula platyphylla* in chemical and spectral data, though its melting point differs from that of compound IV and optical activity is yet unknown.

It is necessary to elucidate the details of the absolute configurations of hannokinin and hirsutanonol on asymmetric carbon in a future study.

Conclusion

In the course of the investigation of the extracts from the wood of Keyamahannoki *Alnus hirsuta* TURCZ. (Betulaceae), nine compounds (named tentatively E-1 to E-9) and vanillic acid were isolated as crystals, and syringic acid was observed on TLC. We proposed the name hannoki ester, hannokinol and hannokinin for the compounds E-2, E-3 and E-4. Four (E-1, E-2, E-3 and E-4) of these nine substances have now been studied in regard to their structure.

From the results of coloration, acetylation, methylation, degradation, hydrolysis and spectral measurement, E-1, hannoki ester, hannokinol and hannokinin could be proved structurally to be trideoxyasadanin-8-ene (I), β -guaiacyl ethyl ferulate (II), 1,7-di-(*p*-hydroxyphenyl)-3,5-diol and 1,7-di-(*p*-hydroxyphenyl)-3-one-5-ol, respectively.

Three (E-1, E-3 and E-4) of above compounds and the other two substances (E-7 and E-8), which were ignored in this paper, belong to the group of diarylheptanoids. As described above, this group has a C₆-C₇-C₆ carbon skeleton and are distributed over the family in nature. Diarylheptanoids are encountered among *Alnus*, *Betula* and *Ostrya* in the same family Betulaceae. The occurrence of these substituents appear to be a characteristic of this family.

The main compounds in Asada *Ostrya japonica* are asadanin and its homologous, of which two aryl groups were coupled at the meta position to the side chain moiety. On the other hand, platyphyllonol in Shirakanba *Betula platyphylla* is not subjected to meta bridged structure. However, the constituents occurring in the wood of Keyamahannoki *A. hirsuta* consist of both types of structure, and this is very interesting from the standpoint of chemotaxonomy and its biochemistry. A considerable difference also exists among various organs of the trees of *Alnus*. An aromatic ring of yashabushiketol and its dihydro-derivative from the buds of Yashabushi *A. firma* and 1,7-diphenyl-heptane-3,5-diol from the leaves of *A. furticosa* and *A. manshurica* was not hydroxylated, whereas that of hannokinol and hannokinin in the wood and of hirsutanonol from the inner bark of *A. hirsuta* was subjected to hydroxylation. Now it will be useful in solving the problem of biosynthesis to ascertain the existence of the compound with the unhydroxylated benzene ring in the leaves of Keyamahannoki. Mono-hydroxylated

compounds are obtained from the wood and di-substituted materials occurred in the inner bark of the same species. Considering the mechanism of hydroxylation in plants, it can be understood that hannokinin and hirsutanonol are formed by alternative path ways of biosynthesis to each other. This seems to be explain that each enzyme system on formation differed between xylem and phloem as a boundary to cambium.

As state above, β -phenylethyl cinnamate, having the structure $(C_6-C_2-CO)-O-C_2-C_6$ from the buds of *A. firma*, has an unhydroxylated benzene ring, but hannoki ester with the same skeleton is subject to hydroxylation and methylation. It has not yet been solved whether hydroxylation and methylation are characteristic of *A. hirsuta* or not. Clearly, it may be considered that the mechanism of biosynthesis differed between the xylem and other organs in tree by the above results.

Finally, it is very interesting with regard to chemosystematics that the occurrence of diarylheptanoids in *Alnus* plays the role of taxonomic tracer. With obtaining information on the structure of the wood constituents, confirmation of the existence of unknown material seems to be the subject for a future study.

Experimental

All melting points were uncorrected. The UV spectra in EtOH solution and in alkali solution were scanned on Hitachi Spectrophotometers EPS-3T and 124, and the IR spectra as KBr disk with a Yanagimoto ISG-1 and a Hitachi Grating Infra red Spectrophotometer 215. The NMR spectra were measured on Hitachi High Resolution NMR Spectrophotometers Model H60B and R-22 with tetramethylsilane as an internal standard, the MS spectra were obtained on a RUN-6 Hitachi Mass Spectrophotometer and a Model Hitachi K-53 GC RMS-4 MS. TLC carried out Kieselgel (nach Stahl); UV lamp, diazo-reagents, ferric chloride and 50% H₂SO₄ as detecting reagents; toluene: ethylformate: formic acid (5:4:1), benzene: acetone (3:1), 50% MeOH as developing solvents. PPC was performed on Toyo Roshi No. 51 and 52 using xylene: dimethylformamide (9:2) and chloroform: EtOH: H₂O (8:2:1, lower layer) as mobile phase. All column chromatography used cellulose powder (Toyo Roshi 100 and 20 mesh: column 5.2×63 cm) and silica gel (Wacogel C-200 and C-300: column 3.8×70 cm). The solvents used were xylene: dimethylformamide (6~15:1) for the cellulose column and benzene: acetone (5~20:1) for the silica gel column.

1. Extraction and Fractionation

In this investigation, the raw material used was collected at Teshio College Experiment Forest, Hokkaido University. Air-dried wood was flecked and milled with a Willey mill through 2 mm screen. The prepared wood meal was stored in polyethylene bag.

Three kg of air-dried sample was in a large percolator with 10 ℓ of 95% EtOH for 72 hrs at room temperature. Then the ethyl alcohol was decanted. This procedure was repeated three times using fresh solvent. About 10 kg of wood meal was treated. The combined ethanolic solution was evaporated under reduced pressure to syrup, and

a portion of syrup for determination was dried by a rotary evaporator (3.6% on oven-dried wood). The ethanolic extract was successively percolated with light petroleum ether, ethyl ether and ethyl acetate in a liquid-liquid extraction apparatus and the yields were 0.4%, 0.6% and 1.5%, respectively. Subsequently, an ether soluble fraction was fractionated with saturated sodium bicarbonate, 10% sodium carbonate, 5% sodium carbonate and 5% potassium hydroxide. The fractions from 65 g of the ether soluble portion were 32.0 g, 22.6 g and 6.4 g as acidic parts, and 3.0 g for neutral part (Fig. 24).

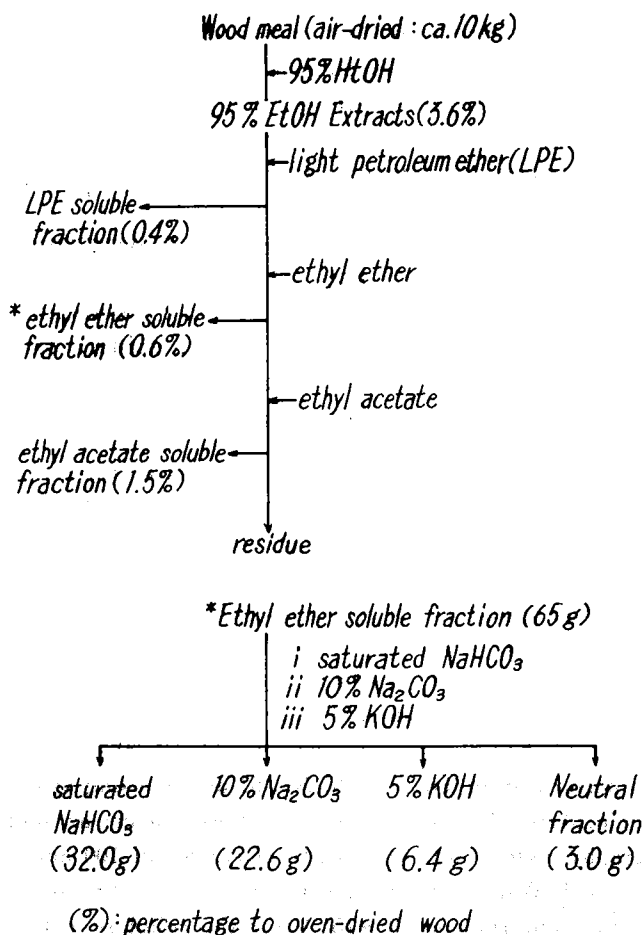


Fig. 24. Separation scheme of extracts from the wood of *Alnus hirsuta* TURCZ.

The 10% sodium carbonate and the 5% potassium hydroxide soluble portion were chromatographed using cellulose and silica gel column, and E-1, E-2, E-5, E-7 and E-8 were fractionated from 10% sodium carbonate soluble portion, and E-3, E-4 and E-6 from 5% potassium hydroxide soluble portion.

2. Isolation of Trideoxyasadanin-8-ene (E-1) (I)

A portion (12 g) of 10% sodium carbonate soluble fraction was placed on the cellulose column and then developed using xylene:dimethylformamide (15:1). One ℓ

of the initial eluates was collected and a crude material was obtained after the removal of the solvent. Several recrystallizations from benzene:acetone (1:1) gave colorless, needles, mp 238~241°C, which were positive to diazo-reagents, ferric chloride and negative to quinone monochlorimide (yield; 0.7 g). UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ : 216, 230, 240 (sh), 297; $\lambda_{\text{max}}^{\text{EtOH-NaOH}}$ m μ : 327; IR $\nu_{\text{max}}^{\text{KBr}}$ cm $^{-1}$: 3,270, 1,675, 1,615, 1,600, 1,505; MS m/e 294 M $^{+}$. Anal. Calcd. for C₁₉H₁₈O₃: C, 77.53; H, 6.16. Found. C, 77.41; H, 6.05.

2-1. Trideoxyasadanin-8-ene diacetate (Ia)

E-1 (148 mg) was set aside with acetic anhydride (2 m ℓ) in dry pyridine (1.6 m ℓ) over night at room temperature. After ice water (100 m ℓ) was poured into the mixture, the resultant precipitate was collected and recrystallized from EtOH to give colorless plates (Ia) (197 mg), mp 213.5~215.0°C. UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ : 230, 235, 267~275; IR $\nu_{\text{max}}^{\text{KBr}}$ cm $^{-1}$: 1,750, 1,685, 1,600, 1,500, 1,375, 1,200; NMR (CDCl₃/60 MHz) τ : 2.90 (1H, d, J=16 Hz), 3.0 (6H, m), 3.61 (1H, d, J=16 Hz), 6.6~7.7 (8H), 7.83 (6H, s). Anal. Calcd. for C₂₃H₂₂O₅: C, 73.00; H, 5.86. Found.: C, 72.65; H, 5.84.

2-2. Hydrogenation of Trideoxyasadanin-8-ene

E-1 (80 mg) was dissolved in EtOH (25 m ℓ) and Pd-C (45 mg) was added. The mixture was hydrogenated over Pd-C for 2 hrs, and then filtrated. The reactant recrystallized from EtOH to give colorless plates (70 mg), mp 224~228°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm $^{-1}$: 3,250, 1,690, 1,600, 1,580, 1,500, 815.

2-3. Oxidation of Trideoxyasadanin-8-ene with KMnO₄

E-1 (140 mg) in dry acetone (40 m ℓ) was refluxed with (Me)₂SO₄ (1.0 m ℓ) and K₂CO₃ (1.4 g) for 30 hrs in a steam bath. After cooling and filtering, the acetone was taken off and the reactant was placed in 5% KOH (100 m ℓ). Oxidation with KMnO₄ was done dropwise and the excess KMnO₄ was degraded with H₂O₂. A mixture was passed through filters and the filtrate was extracted by ethyl acetate. After being treated with Na₂SO₄, the removal of solvent yielded the oxidative product, mp > 300°C. From the behaviour on TLC and PPC, this compound was indentified as 2, 2'-dimethoxy-5, 5'-dicarboxybiphenyl.

3. Isolation of β -Guaiacyl ethyl ferulate (E-2) (Hannoki ester) (II)

Eluate No. 710~804 from the cellulose column was collected and then the solvent was removed to give a syrup. A cream-colored material was precipitated on the addition of water. After several recrystallizations from EtOH, a crude solid gave a pure E-2 as colorless needles, mp 194~195°C, positive to diazo-reagents and ferric chloride. UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ : 219, 233, 291, 329; $\lambda_{\text{max}}^{\text{EtOH-NaOH}}$ m μ : 250, 301, 380; IR $\nu_{\text{max}}^{\text{KBr}}$ cm $^{-1}$: 3,397, 1,700, 1,620, 1,600, 1,500, 1,290, 1,150; MS: m/e 344M $^{+}$. Anal. Calcd. for C₁₉H₂₀O₆: C, 66.27; H, 5.85; OCH₃: 17.98. Found.: C, 66.23; H, 5.99; OCH₃: 18.03 (yield: 0.7 g).

3-1. Hannoki ester diacetate (IIa)

(E-2) (100 mg) with acetic anhydride (2 m ℓ) in dry pyridine (2 m ℓ) was kept over night at room temperature. Then a mixture was poured into ice water (100 m ℓ) and the resultant precipitate was collected. The amorphous diacetate (126 mg) obtained after recrystallization from 60% EtOH had mp 70~73°C. UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ : 216, 225 (sh), 281, 310; IR $\nu_{\text{max}}^{\text{KBr}}$ cm $^{-1}$: 1,760, 1,700, 1,630, 1,600, 1,500, 1,370, 1,200; NMR (CDCl₃/60 MHz) τ : 2.31 (1H, d, J=13 Hz), 2.88~3.27 (6H, m), 3.60 (1H, d, J=13 Hz), 5.52~5.75 (2H),

6.11 (3H, s), 6.23 (3H, s), 7.10~7.34 (2H), 7.68 (6H, s). Anal. Calcd. for $C_{23}H_{24}O_3$: C, 64.48; H, 5.65. Found.: C, 65.08; H, 5.72.

3-2. Oxidation of Hannoki ester with $KMnO_4$

E-2 (50 mg) in a small portion of EtOH was reacted with an ether solution of excess CH_2N_2 . The methylated E-2 obtained was dissolved immediately in 5% KOH and then 3.5% $KMnO_4$ was added dropwise. Thereafter the excess $KMnO_4$ was treated with H_2O_2 and the resulting MnO_2 was taken off. The filtrate was then acidified with HCl and continued to be extracted by ethyl ether. The reactant obtained was identical to an authentic specimen of veratric acid. mp $180^\circ C$.

3-3. Alkalline hydrolysis of Hannoki ester

E-2 (0.5 g) in 3% ethanolic KOH (40 ml) was refluxed in a steam bath for 3 hrs. After cooling, the solvent was evaporated under reduced pressure and 40 ml of water was added. The aqueous solution of the reactant was acidified with HCl and extracted by ethyl ether (100 ml). In order to obtain an acidic portion, the ether solution was fractionated by 5% $NaHCO_3$ (20 ml). When the $NaHCO_3$ fraction was acidified with HCl, a crude solid was precipitated and collected. Several recrystallizations from 50% EtOH yielded Hydrolysate I (228 mg) as colorless needles, mp $174\sim 175^\circ C$. It was positive to diazo-reagents and ferric chloride. UV λ_{max}^{EtOH} m μ : 236, 298, 323; $\lambda_{max}^{EtOH-NaOH}$ m μ : 240, 308, 349; IR ν_{max}^{KBr} cm^{-1} : 3,430, 1,685, 1,660, 1,600, 1,500. The chemical and spectral natures were identical to that of an authentic specimen of ferulic acid. The mixed mp was undepressed.

The neutral component was yield as a viscous material (Hydrolysate II) (ca. 190 mg), and was treated with excess CH_2N_2 . The NMR spectrum of the methyl ether revealed the following signals: 3.22 (3H, m), 6.17 (6H, 2H), 7.25 (2H, t, $J=7$ Hz), 8.37 (1H, s). This result agreed very well with that of synthetic homoveratryl alcohol.

3-4. Hannoki ester dimethyl ether (IIb)

E-2 (30 mg) was suspended in a small portion of dry ethyl ether and then an ethyl ether solution of excess CH_2N_2 was added. After the removal of the solvent, a crude methylate was obtained in a faint yellow oily state. By recrystallization from MeOH, dimethyl ether of E-2 was yielded as colorless needles (18 mg), mp $123.9^\circ C$. Anal. Calcd. for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50. Found.: C, 67.97; H, 6.60. MRN ($CDCl_3/90$ MHz) τ : 2.37 (1H, d, $J=16$ Hz), 2.88~3.32 (6H, m), 3.71 (1H, d, $J=16$ Hz), 5.63 (2H, t, $J=7$ Hz), 6.12 (6H, s), 6.18 (6H, s), 7.12 (2H, t, $J=7$ Hz).

3-5. Synthesis of Homoveratryl alcohol

Veratrum aldehyde (5 g), dry hippuric acid (6 g), fused sodium acetate (2.5 g) in acetic anhydride (9 ml) was heated in an oil bath at $110^\circ C$ until it had melted and discolored to a deep yellow. Then the oil bath was replaced by a water bath and heating continued for 2 hrs. After cooling, ethanol (40 ml) was added slowly into the mixture, and it was set aside over night at room temperature. The precipitated yellow crystals were filtered, washed with ice-cold ethanol (3 ml) and hot benzene (3 ml) twice. Then 2-phenyl-4-veratral-5-oxazolone was yielded, mp $149\sim 150^\circ C$ (yield: 6.4 g). The 2-phenyl-4-veratral-5-oxazolone (6 g) in 10% NaOH (34 ml) was refluxed in an oil bath until the end of NH_3 generation. 40% NaOH (5 ml) was added to the reactant and subsequently

fed $\text{H}_2\text{O}_2\text{-H}_2\text{O}$ (1:1) (9 ml) cooling ice-NaCl. After being kept over night and being acidified with conc. HCl (14.4 ml), the mixture was immediately extracted with hot benzene (20 ml and 30 ml twice). The benzene solution was dried by MgSO_4 , and evaporated. The residue with 30 ml of MeOH containing conc. H_2SO_4 (0.3 ml) was refluxed for 5 hrs. After MeOH was taken off, ice-water (15 ml) was fed in the mixture and shaken. Again, the mixture was extracted with benzene (3 ml and 10 ml twice) and the benzene soluble portion was then washed with 10% Na_2CO_3 (3 ml) twice, water (3 ml) twice, and dried by MgSO_4 . The resultant material was distilled under reduced pressure, and homoveratric acid methyl ester was obtained at $176\sim 178^\circ\text{C}$ under 16 mmHg¹³⁾. Yield 2.5 g.

Homoveratric acid methyl ester (2 g) in dry tetrahydrofuran (30 ml) was treated with LiAlH_4 (1 g) in dry tetrahydrofuran (50 ml) by stirring for 6 hrs. Cooling at 0°C , a mixture of tetrahydrofuran: H_2O (15:2 v/v) was carefully added to the reactant and set aside for 2 hrs. After the evaporation of the solvent, the residue was extracted with ethyl acetate (50 ml) and ethyl acetate soluble portion dried by Na_2SO_4 . After being removed the solvent, homoveratryl alcohol was obtained as viscous solid. Yield ca. 1.5 g. NMR ($\text{CDCl}_3/90\text{ MHz}$) τ : 3.25 (3H, m), 6.17 (6H, s), 6.22 (2H, t, $J=7\text{ Hz}$), 7.22 (2H, t, $J=7\text{ Hz}$), 8.22 (1H, s). mp $37\sim 40^\circ\text{C}$. Anal. Calcd. for $\text{C}_{10}\text{H}_{14}\text{O}_3$: C, 65.91; H, 7.74. Found.: C, 65.19; H, 7.69.

3-6. Synthesis of Hannoki ester dimethyl ether (IIIa)

The synthesis of 3,4-dimethoxycinnamic acid has been carried out by a method of Adams, R.¹⁴⁾ Veratrum aldehyde (3 g), malonic acid (4 g), anilline (0.1 ml) were dissolved in dry pyridine (2 ml) and heated in a steam bath at 55°C for 10 hrs. The reactant was poured into water (50 ml) and the precipitate collected. After several recrystallizations from EtOH, 3,4-dimethoxycinnamic acid was yielded as colorless needles, mp 183°C . Yield 2.5 g. Anal. Calcd. for $\text{C}_{11}\text{H}_{12}\text{O}_4$: C, 63.45; H, 5.81. Found.: C, 63.13; H, 5.89.

The above compound (2 g) was heated with thionyl chloride (5 g) in a steam bath for 30 min.. Then excess thionyl chloride was taken away under reduced pressure and the oily material obtained became a solid by cooling. This solid was used for next process without purification.

The synthesis of ester IIb was carried out using metal halide according to Hill, M.¹⁵⁾ 3,4-Dimethoxycinnamic acid chloride (1.3 g) in 10 ml of carbon tetrachloride was mixed with homoveratryl alcohol (2 g) at room temperature. To this solution was added 0.5 g of crushed anhyd. AlCl_3 . After the initial surge of hydrogen chloride gas had subsided, the reaction was warmed to reflux and held 30 min. to complete the reaction. At the end of this period, the evolution of gas was virtually nil. Upon cooling, the mixture set to a mass of crystals which were filtered off. The solid was then slurried with dil. HCl, filtered, slurried with 5% NaHCO_3 , filtered and dried. Recrystallization from EtOH gave 3.2 g of hannoki ester dimethyl ether, mp 124.3°C . Anal. Calcd. for $\text{C}_{21}\text{H}_{24}\text{O}_6$: C, 67.73; H, 6.50. Found.: C, 67.45; H, 6.68. NMR ($\text{CDCl}_3/90\text{ MHz}$) τ : 2.38 (1H, d, $J=16\text{ Hz}$), 2.90~3.32 (6H, m), 3.72 (1H, d, $J=16\text{ Hz}$), 5.60 (2H, t, $J=7\text{ Hz}$), 6.11 (6H, s), 6.17 (6H, s), 7.05 (2H, t, $J=7\text{ Hz}$).

4. Isolation of 1,7-di-(p-hydroxyphenyl)-heptane-3,5-diol (E-3) (Hannokinol) (III)

5 g of 5% KOH soluble fraction was placed on a silica gel column (3.8×70 cm) and then was developed using benzene:acetone (20~1:1) as mobile phase. Eluate No. 652~752 was collected and concentrated. The solid obtained was recrystallized from benzene:acetone (1:1) to give colorless plates (1.5 g), mp 165~166°C, $[\alpha]_D^{25} = +32.5$ (c=0.53 in EtOH), and was positive to diazo-reagents, ferric chloride, alkali-alkyl xanthate, and negative to quinone monochlorimide. UV $\lambda_{\max}^{\text{EtOH}}$ m μ : 225, 279.5, 286 (sh); $\lambda_{\max}^{\text{EtOH-NaOH}}$ m μ : 227.5, 243, 289, 300; IR ν_{\max}^{KBr} cm⁻¹: 3,400, 3,270, 1,600, 1,508, 1,110, 840, 800. MS m/e: 316 M⁺, 298 (M-18), 280 (M-36), 173, 160, 150, 149, 133, 121, 120, 107 (base ion), 91, 77, 65, 51, 39. Anal. Calcd. for C₁₉H₂₄O₄: C, 72.12; H, 7.65. Found.: C, 72.48. H, 7.68.

4-1. Hannokinol tetraacetate (IIIa)

E-3 (50 mg) with acetic anhydride (1.0 ml) in dry pyridine (1.0 ml) was set aside over night at room temperature. On being poured into 100 ml of ice-water, the mixture gave an oily material. In order to purify it, the oily compound was treated with ethyl ether, but failed to crystallize. After the removal of ethyl ether, the reactant dried on P₂O₅ under reduced pressure for one week and gave a faint yellow, viscous tetraacetate. NMR (CDCl₃/90 MHz): 2.79~3.08 (8H, m), 5.02 (2H, quin, J=7 Hz), 7.43 (4H, t, J=7 Hz), 7.78 (6H, s), 8.04 (6H, s), 8.19 (6H, m).

4-2. Oxidation of methylated Hannokinol With KMnO₄

Compound III (43 mg) was methylated with CH₂N₂ in the usual procedure. The reactant was immediately dissolved 5% KOH (40 ml) and subsequently oxidized with 3.5% KMnO₄ accordance with 2-3 (experimental). Recrystallization from 60% EtOH gave a crystalline material, mp 183°C. The mixed mp with an authentic specimen of p-anisic acid was undepressed.

5. Isolation of 1,7-di-(p-hydroxyphenyl)-heptane-3-one-5-ol (E-4) (Hannokinin) (IV)

Eluate No. 343~599 of the 4 (experimental) column was collected and evaporated. A crude solid was recrystallized from benzene:acetone (1:1) to give colorless needles, mp 131~132°C, $[\alpha]_D^{25} = +20.7$ (c=0.72 in EtOH). It gave a positive color test diazo-reagents, ferric chloride, 2,4-dinitrophenylhydrazine, alkali-alkyl xanthate, and negative to quinone monochlorimide. UV $\lambda_{\max}^{\text{EtOH}}$ m μ : 225, 280, 285, 288 (sh); $\lambda_{\max}^{\text{EtOH-NaOH}}$ m μ : 246, 288, 298; IR ν_{\max}^{KBr} cm⁻¹: 3,400, 3,270, 1,690, 1,600, 1,500, 1,110, 840, 800. MS m/e: 314 M, 296 (M-18), 202, 190, 176, 175, 164, 150, 149, 121, 107 (base ion), 94, 91, 77, 65, 51, 43, 39. Anal. Calcd. for C₁₉H₂₂O₄: C, 72.59; H, 7.05. Found.: C, 72.78; H, 7.18.

5-1. Hannoeinin triacetate (IVa)

E-4 (0.2 g) with acetic anhydride (2 ml) in dry pyridine (2 ml) was kept over night at room temperature. When the mixture was poured into 100 ml of ice-water, the reactant was oily. In a similar manner as with hannokinol tetraacetate, the oily material dried on P₂O₅ under reduced pressure to give a pale yellow, viscous substance. NMR (CDCl₃/90 MHz) τ : 2.93 (8H, m), 4.75 (1H, quin, J=7 Hz), 7.23~7.46 (8H, m), 7.77 (6H, s), 8.06 (3H, s), 8.16 (2H, m).

5-2. Oxidation of methylated hannokinin with KMnO₄

As with methylated hannokinol, E-4 (0.3 g) was treated with CH₂N₂ and subse-

quently oxidized with KMnO_4 . The substance obtained had mp $182\sim 184^\circ\text{C}$ and was identified with an authentic specimen of p-anisic acid on TLC and mixed mp.

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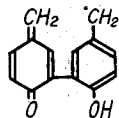
要 約

ケヤマハンノキ *Alnus hirsuta* TURCZ. (Betulaceae) の材の抽出成分に関する知見は未だ得られていないので、材中の成分を検索する目的で、本研究が行われた。その結果、アルコー

ル抽出物中、エーテル可溶部の酸性部から9種の結晶性物質(仮りにE-1からE-9と記す)と vanillic acid を単離し、さらに syringic acid を TLC 上で確認した。このE-系列の中、E-2、E-3 および E-4 は、その化学的性状から未だ知られていない物質と考えられ、それぞれ Hannoki ester, Hannokinol および Hannokinin と命名した。この報告では主に、E-1、E-2、E-3 および E-4 の化学構造についての結果を報告する。

1. E-系列の化合物はそのUV、IR、MSスペクトルおよび酸化分解物の性状から三つの群に大別することができる。第一群はE-3、E-4 および E-9 の化合物からなり、IRスペクトルで $840\sim 800\text{ cm}^{-1}$ に1,4-置換芳香核に由来する吸収帯を示めし、これら化合物のメチル化合物を過マンガン酸カリで分解すると *p*-anisic acid を与える。このことはMSスペクトルで $m/e\ 107$ (base ion) $((\text{HO})\text{Ar}-\text{CH}_2\cdot)^+$ を与えることから支持される。

第二群の化合物はそのUVスペクトルで第一群より長波長側の $300\sim 310\text{ m}\mu$ 附近に極大値をもち、共役系が強められている。メチル化合物の分解で2,2'-dimethoxy-5,5'-dicarboxy-biphenyl を与え、部分構造としてビフェニル核をもっている。

このことはMSスペクトルで $m/e\ 211$  のイオンピークを示すことによって

理解できる。この群にはE-1、E-5、E-7 および E-8 が属する。

第三群は残りのE-2 および E-6 を含み、第一群および第二群より共役系が強められ構造を有することが、UVスペクトル上で判断できる。

2. Tirdeoxyasadanin-8-ene (E-1) (I)

第二群に属する化合物で、分子式 $\text{C}_{19}\text{H}_{18}\text{O}_3$ ($m/e\ 294\text{ M}^+$)、m.p. $238\sim 241^\circ\text{C}$ をもち、呈色反応からフェノール性水酸基の存在が確認される。分子式中の酸素原子の1個はIRスペクトルより α,β -不飽和カルボニルとして存在し、これはフェニル核と共役していない。メチル化合物の分解、MSスペクトルから構造中にビフェニル核を有していることが認められた。不飽和結合の存在は、Pd-Cによる接触還元で、IRスペクトル上の不飽和結合に由来する $1,615\text{ cm}^{-1}$ の吸収帯の消滅およびカルボニル基の吸収帯の移動 ($1,675\text{ cm}^{-1}\rightarrow 1,690\text{ cm}^{-1}$) で説明できる。既知化合物の性質との比較により、E-1の構造は tirdeoxyasadanin-8-ene に相当し、アサダ材からの物質と一致した⁵⁾。従ってE-1の炭素骨格は $\text{C}_6-\text{C}_7-\text{C}_6$ の diarylheptanoids に相当し、さらに meta bridged biphenyl 構造を有する。

3. Hannoki ester (β -Guaiacylethyl ferulate) (E-2) (II)

E-2は第三群に属し、UV、IR、MS、加水分解物の性状、メチル化合物の分解物およびアセテートのNMRスペクトル等から ferulic acid と homovanillyl alcohol とから成るエステル β -guaiacylethyl ferulate であることを既に速報とし発表した⁶⁾。ここでは、さらにその構造の確認のためにメチル化合物の性状および合成によって構造を検討した。

E-2 のジメチルエーテル, m.p. 123.9°C, NMR のスペクトルから既報の結果が正しいことを確認した。さらに hannoki ester のジメチルエーテルを合成して確めるために, homoveratryl alcohol を合成し, ついで 3,4-dimethoxy cinnamic acid chloride と反応させて合成品を調製した。

この物質は融点 124.3°C で, E-2 のメチル化で得た物質との混融試験ではその融点は降下しなかった。両者の NMR スペクトルは一致した。この結果より, E-2 は C_6-C_3 および C_6-C_2 の炭素骨格をもつエステル β -guaiacylethyl ferulate であるとの以前の報告は正しかった。この種の化合物として芳香核が水酸基置換およびメトキシ基置換されていない化合物 β -phenylethyl cinnamate がヤシャブシ *Alnus firma* から得られていることは甚だ興味深い。

4. Hannokinol (1,7-Di-(*p*-hydroxyphenyl)-heptane-3,5-diol) (E-3) (III)

この物質は E-4 と共に第一群に属する。分子式は $C_{19}H_{24}O_4$ (m/e 316 M^+), m.p. 165~166°C を示めし, 光学活性である ($[\alpha]_D^{25} = +32.5$)。呈色反応からフェノール性および二級アルコール性水酸基の存在, さらに UV, IR スペクトルからその構造中に *p*-hydroxyphenyl 核をもつことが確認された。メチル化物の分解物が *p*-anisic acid であったことは, このことを支持する。E-3 の tetraacetate の NMR スペクトルから, 2 個の *p*-hydroxyphenyl 核に由来する 8 個のプロトン, 2 個のフェノール性および 2 個の二級アルコール性水酸基の存在が確認された。さらに 2 個のベンジル基に由来するメチレンおよびアルコール性水酸基に隣接する 3 個のメチレン基が帰属される。その他ヘプタン炭素鎖上の 2 個の水酸基が置換された炭素上のプロトンがそれぞれ確認される。

これらの結果を総合すると, E-3 の炭素骨格は $C_6-C_7-C_6$ の diarylheptanoids に相当し, その構造は 1,7-di-(*p*-hydroxyphenyl)-heptane-3,5-diol と決定された。

この構造に類似したものは *A. fruticosa* および *A. manshurica* の葉から得た 1,7-diphenyl-heptane-3,5-diol が知られており³⁾, この物質の化学的知見と E-3 の化学的性質は矛盾しない。

5. Haunokinol (1,7-Di-(*p*-hydroxyphenyl)-heptane-3-one-5-ol) (E-4) (IV)

E-4, 分子式 $C_{19}H_{22}O_4$ (m/e 314 M^+), m.p. 131~132°C, は E-3 と同様, 第一群に属し, 光学的に活性である ($[\alpha]_D^{25} = +20.7$)。2,4-DNHP および alkali-alkyl xanthate 反応が陽性であり, この物質は E-3 と異なり ketol の構造をもつ。UV, IR, MS, triacetate の NMR スペクトルから E-3 の部分構造に一致し, さらにメチル化物を過マンガン酸カリ酸化すると *p*-anisic acid を生ずることから支持される。

E-3 および yashabushi ketol とそのジヒドロ体の化学的性質の比較から, E-3 と同様, $C_6-C_7-C_6$ の炭素骨格をもつ diarylheptanoid であり, その構造は 1,7-di-(*p*-hydroxyphenyl)-3-one-5-ol と決定した。

この研究の進行中, 寺沢らはシラカンバ *Betula platyphylla* の内皮から diarylheptanoid

の platyphyllgnol を単離した⁴⁾。この物質、分子式 $C_{19}H_{22}O_4$ (m/e 314 M^+), m.p. 125~126°C, の化学的性状およびスペクトルの結果は、全く E-4 と一致し、従って E-4 はこの platyphyllgnol と構造的には同じと考えて差支えない。しかし融点が異なり、旋光度が未だ不明なので、この点についての検討は今後残された課題である。

trideoxyasadanin-8-ene の環化した構造の物質を含め、*Alnus* species に diarylheptanoids の生起は興味のあるところである。カバノキ科のアサダ材からは環化した物質、シラカンバ内皮、およびその他のハンノキ属から環化しない構造の物質が存在する。ケヤマハンノキ材はこの両者のタイプの化合物が存在し、又同じハンノキ属でも部位によって水酸基置換の様相が異なっている。これら化合物の生合成的解明は今後の問題であるが、chemosystematics の立場と共に甚だ興味のある課題である。