BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 2223—2229 (1970)

Chemical Constituents of *Alnus firma* (BETULACEAE). I. Phenyl Propane Derivatives Isolated from *Alnus firma*

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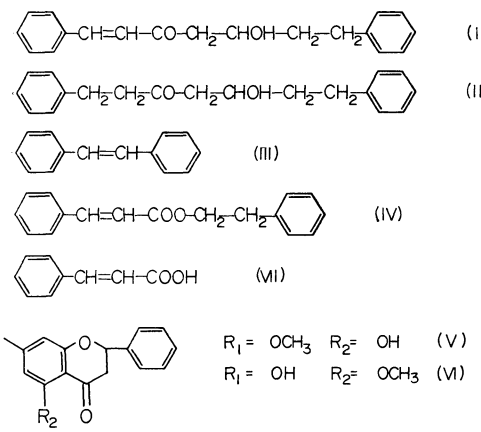
(Received February 2, 1970)

Viscous substances extracted from young shoots of *Alnus firma* were investigated, and two new hydroxyketones, named yashabushiketol (I) and dihydroyashabushiketol (II), *trans*-stilbene (III), β-phenylethyl cinnamate (IV), pinostrobin (V), alpinetin (VI), cinnamic acid (VII), paraffin hydrocarbons (*n*-, anteiso and iso) and triglycerides were identified on the basis of the chemical and spectral data. The biosynthetic pathways of the above aromatic compounds were discussed.

Alnus firma (the Japanese name is Yashabushi), which grown on poor and waste land, is a deciduous plant and a member of the birch-tree family (BETULACEAE). It is known that this plant can be used as an erosion-control plant and that its young shoots (Catkin) and leaves secrete viscous substances, late in February in Japan. No reports on the chemical constituents of *Alnus* genera have yet been presented, though. In the course of the investigation of the benzene extract of the viscous material on the young shoots, two new hydroxyketones, which possess a phenyl propane skeleton, were isolated, together with five related aromatic compounds. The present communication will be concerned with the structure elucidation and the biosynthetic pathways of these seven aromatic compounds, and with the detection of paraffin hydrocarbons and triglycerides.

Results and Discussion

The extraction of the young shoots with benzene gave a dark green, viscous material. After the removal of the acidic and phenolic constituents, the neutral portion was fractionated into three parts by silica-gel chromatography using *n*-hexane, benzene, and ethyl acetate as the eluting agents.



Yashabushiketol (I). 1,7-Diphenyl-5-hydroxy-1-hepten-3-one. Compound (I) was isolated as colorless needles by ethyl acetate extraction from the fractions. This compound (mp 59.5—60.5°C (from *n*-hexane), $[\alpha]_D^{20}$ +29.0°, R_f=0.14 on TLC) gives positive coloration with 2,4-DNP and with alkali-alkyl xanthate. The molecular formula of compound (I) was established as $C_{19}H_{20}O_2$ from a high-resolution mass spectrum. The UV spectrum shows λ_{max}^{EtOH} 217 mμ (log ε, 4.38), 227 (3.90) and 289 (4.18), suggesting the presence of cinnamoyl chromophore (Ar–CH=CH–CO).[1] The IR spectrum shows absorption bands at 1703 cm^{-1} (α,β-unsaturated carbonyl group), 3090, 3050, 1607, 1490, 1447, 752, and 702 cm^{-1} (monosubstituted benzene-ring), 3400 and 1100 cm^{-1} (secondary hydroxyl group), 1410 cm^{-1} (active methylene), and 3025, 1678 and 980 cm^{-1} (*trans* double bond). The NMR spectrum of I contains the following signals: a typical AB-type doublet at 7.61 and 6.71 ppm (J_{AB}=16 Hz, 1H, each) assignable to *trans* ethylenic protons of the cinnamoyl group, two overlapped singlets, at 7.27 and 7.25 ppm, accounted for by two monosubstituted benzene-ring protons (10H), a quintet at 4.07 ppm (J=7 Hz, 1H of –CH–) attributable to the presence of the $-CH_2-CHOH-CH_2-$ grouping, a complex multiplet located between 2.70 and 2.90 ppm (2H, of $-CH_2-$) assignable to the presence of the $-CO-CH_2-CHOH-$ grouping,[2,3] a triplet at 2.60 ppm (J=7 Hz, 2H of Ar–CH_2-) due to the Ar–CH_2–CH_2- group, a quartet at 1.89 ppm (J=7 Hz, 2H of –CH–CH_2–CH_2-) due to the –CHOH–CH_2–CH_2- group, and a broad singlet at 3.20 ppm (1H), which disappeared on

1) A. I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon Press, Oxford (1964), p. 115.

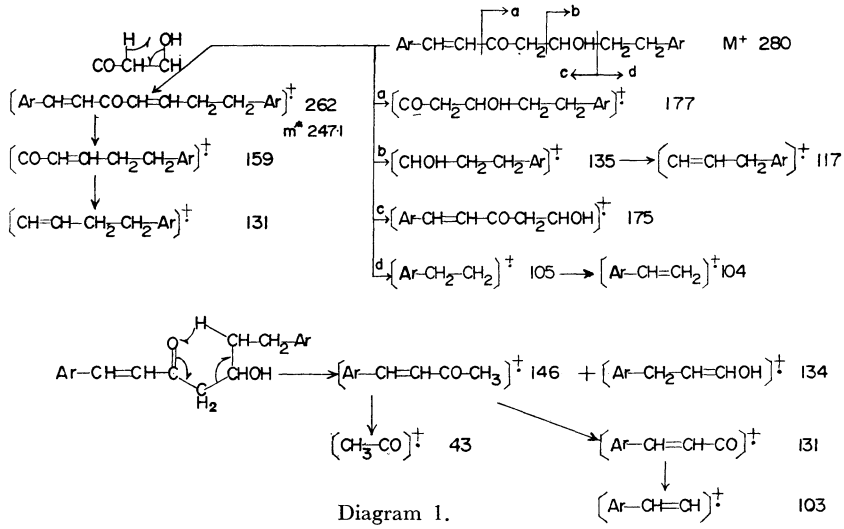
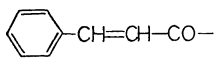
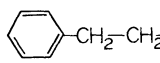
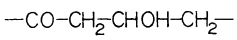


Diagram 1.

equilibration with D_2O, assignable to a hydroxy group. When I in pyridine was treated with acetic anhydride, a yellow, viscous monoacetate was obtained (IR: 1735, 1239 and 1028 cm^{-1}). That a hydroxyl group of I was secondary was proved by the fact that 4.07 ppm (quin. $J=7$ Hz, 1H) was shifted to 5.40 ppm (quin. $J=7$ Hz, 1H) after the acetylation of I.[4] Ketol (I) contains one double bond, as it absorbed one mole equivalent of hydrogen on catalytic hydrogenation over an Adams' catalyst in acetic acid and produced a dihydroderivative, $C_{19}H_{22}O_2$ (M^+ 282), mp 36.0—37.0°C, $R_f=0.25$. The IR spectrum of the dihydroderivative does not exhibit absorption bands at 3025, 1678, 1574, and 980 cm^{-1}, nor could the two doublet signals of the NMR spectrum at 7.61 and 6.71 ppm be seen. From the above chemical and spectral evidence, the following partial structures may be suggested. The ketol (I) contains the molecular







ion at *m/e* 280 ($C_{19}H_{20}O_2$ from the high-resolution mass measurements), the base peak at *m/e* 91 corresponding to a tropyrium ion, and a prominent ion corresponding to M−18 (*m/e* 262), all of which evidence indicates I to be an alcohol. The presence of characteristic ions, *m/e* 39, 51, 65, and 77 indicates I to be a monosubstituted benzene derivative. Other strong ions (*m/e* 177, 175, 159, 146, 134, 117, 105, 104, 103 and 43) in the mass spectrum may be explained as is represented in Diagram 1. The presence of the fragment ions at *m/e* 146, 134, 131, and 103 is understandable, since I, possessing a γ-hydrogen atom for a carbonyl group, undergoes β-cleavage with hydrogen transfer and the product (280→146+134) of β-cleavage is degradated by the loss of a methyl radical (146→131) and of carbon monoxide (131→103) successively. In the mass spectrum of the hydrogenated product of I, the strength of the fragment ions at *m/e* 131 decreased and the fragment of *m/e* 262 disappeared, while *m/e* 133 $(Ar\text{-}CH_2\text{-}CH_2\text{-}CO)^+$ and *m/e* 264 (M—18) newly appeared. On the basis of the above-mentioned chemical and spectral data, the structure of I was determined to be 1,7-diphenyl-5-hydroxy-1-hepten-3-one (I). We proposed the name "yashabushiketol" for this compound in a preliminary report.[5]

Dihydroyashabushiketol (II). 1,7-Diphenyl-5-hydroxy-3-heptanone. The high-resolution mass measurement of the II isolated from the ethyl acetate fraction clearly established its molecular formula as $C_{19}H_{22}O_2$. This compound, (II) (M^+ 282, mp 36.0—37.0°C (colorless needles from *n*-hexane), $R_f=0.25$), gives positive coloration with 2,4-DNP and with alkali-alkyl xanthate, and negative coloration with ferric chloride, ferric hydroxamate, and Tollens' reagent. Compound (II) is shown by its spectral properties to possess a simple ketone group

2) R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds," John Wiley & Sons, Inc., New York (1967), p. 129.

3) G. Rücker, *Chem. Ber.*, **102**, 2707 (1969).

4) W. Herz, K. Ueda and S. Inayama, *Tetrahedron*, **19**, 483 (1963).

5) Y. Asakawa, F. Genjida, S. Hayashi and T. Matsuura, *Tetrahedron Lett.*, **1969**, 3235.

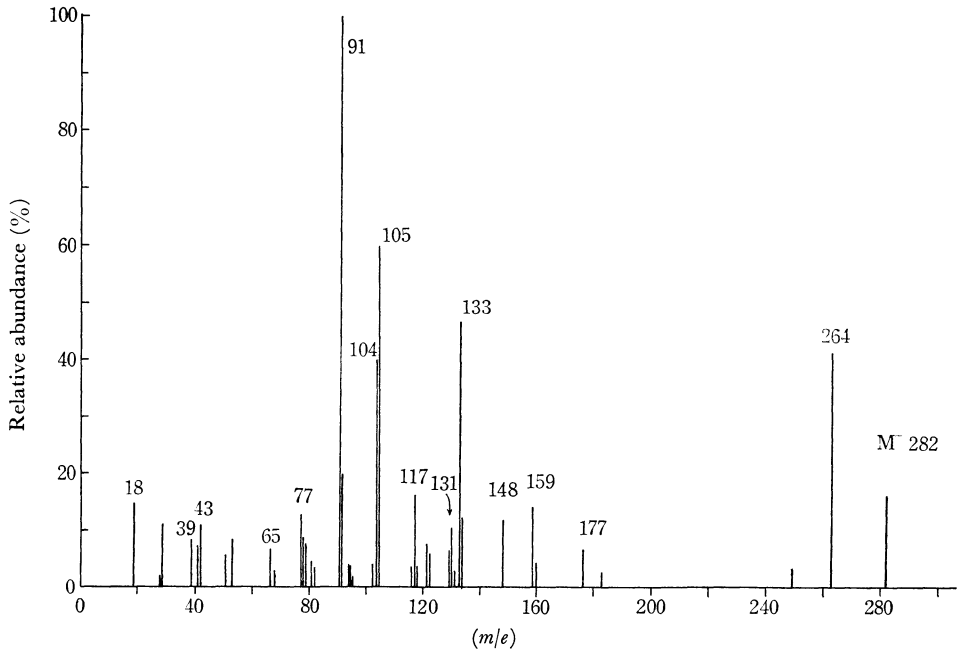


Fig. 1. Mass spectrum of dihydroyashabushiketol (1,7-diphenyl-5-hydroxy-3-heptanone).

(λ_{max}^{EtOH} 285 mμ (log ε, 2.08), ν_{max}^{liq} 1706 cm^{-1}), a mono-substituted benzene-ring (ν_{max}^{liq} 3060, 3020, 1600, 1490, 1450, 745 and 698 cm^{-1}), a non-conjugated benzene-ring (λ_{max}^{EtOH} 248 mμ (log ε, 2.50), 254 (2.53), 261 (2.54) and 268 (2.44)), active methylene (1409 cm^{-1}), and a secondary hydroxyl group (3440 and 1100 cm^{-1}). The NMR spectrum of II in $CDCl_3$ indicates signals at 7.22 ppm (s, 10H, two mono-substituted benzene-ring protons), 4.07 ppm (quin. J=7 Hz, 1H, $-CH_2-C\underline{H}OH-CH_2-$), 3.10 ppm (s, 1H, O$\underline{H}$), complex signals located between 2.70 and 2.90 ppm (4H, $-C\underline{H}_2-CO-C\underline{H}_2-CHOH-$), 2.55 ppm (two triplets partially overlapped, J=7 Hz, 4H, two $Ar-C\underline{H}_2-CH_2-$) and complex multiplets located between 1.55 and 1.85 ppm (2H, $-CHOH-C\underline{H}_2-CH_2-$). The mass spectrum (Fig. 1) of II is closely analogous to that of yashabushiketol (I). It shows the parent ion at *m/e* 282, and the base peak at *m/e* 91 corresponding to that of a tropyrium ion. An important difference between the fragmentations of these two ketols, (I) and (II), is that II possesses the molecular ion at *m/e* 282 and fragment ions at *m/e* 264 (M − 18), *m/e* 148 $(Ar-CH_2-CH_2-CO-CH_3)^+$, and *m/e* 133 $(Ar-CH_2-CH_2-CO)^+$, whereas I has the molecular ion at *m/e* 280 and fragment ions at *m/e* 262 (M − 18), *m/e* 146 $(Ar-CH=CH-CO-CH_3)^+$, and *m/e* 131 $(Ar-CH=CH-CO)^+$. As II has only two larger fragments and the molecular ion more than I, judging from a comparison between the mass spectra of two compounds, it seems that ketol (II) is the dihydroderivative of I. Other strong fragment ions also appear at *m/e* 177 $(Ar-CH_2-CH_2-CO-CH_2-CH=OH)^+$, *m/e* 159 $(Ar-CH_2-CH_2-CO-CH=CH)^+$ or $(Ar-CH_2-CH_2-CH=CH-CO)^+$, *m/e* 131 ($C_{10}H_{11}^+$, $(Ar-CH_2-CH_2-CH=CH)^+$ from high-resolution mass measurement), *m/e* 117 $(CH=CH-CH_2-Ar)^+$, and *m/e* 43 $(CH_3-CO)^+$. The peaks at *m/e* 39, 51, 65 and 77 constitute additional evidence for the benzene ring. These results have shown the structure of this ketol to be 1,7-diphenyl-5-hydroxy-3-heptanone (II). Furthermore, this structure was confirmed by the fact that the TLC R_f value, the mp, and the IR spectrum of II were all completely identical with those of the hydrogenated product of yashabushiketol (I). We proposed the name "dihydroyashabushiketol" for this compound in a previous communication to this bulletin.[6]

***trans*-Stilbene (III).** Colorless leaflet crystals, M^+ 180, mp 120.0—121.0°C, R_f=0.75, were isolated from the *n*-hexane extract. The melting point, R_f value, UV, IR, and NMR spectra of this isolated compound (III) were the same as the authentic *trans*-stilbene. There has been no report concerning the natural occurrence of *trans*-stilbene, except that Kacheler (1869) suggested the existence of stilbene on the basis of its melting point in Perubalsam oil.[7]

β-Phenylethyl Cinnamate (IV). Colorless needles (M^+ 252, mp 56.0—57.0°C, R_f=0.50, positive coloration with ferric hydroxamate) were isolated in a pure state (one peak, NGS 10%, 212°C) from the benzene-eluted fraction of the extract. The alkaline hydrolysis of the isolated crystals (IV) gave cinnamic acid and β-phenylethyl alcohol. The

6) Y. Asakawa, This Bulletin, **43**, 575 (1969).

7) E. Gildmeister, "Die Ätherischen Öle," Academie-Verlag, Berlin (1960), p. 413.

UV, IR and NMR spectra, the mp, the R_f value and the GLC Rt of this compound (IV) were all completely identical with those of the authentic β-phenylethyl cinnamate. It was clarified that the essential constituent with a pleasant smell in the yound shoot of *A. firma* is β-phenylethyl cinnamate, and that this compound gives rise to the viscosity of the young shoots. The only report regarding the finding of β-phenylethyl cinnamate from *Populus balsamifera* has been made by Goris and Canal.[8]

Pinostrobin (5-Hydroxy-7-methoxy-flavanone) (V). Compound (V) was isolated from the benzene-eluted fraction as colorless leaflet crystals (M^+ 270, mp 99.0—100.0°C, R_f=0.48, positive coloration with ferric chloride and with magnesium-hydrochloric acid). The mass spectrum of V is closely analogous to that of 5,7-dihydroxy flavanone.[9] When V in pyridine was treated with acetic anhydride, it gave a mono-acetate. On methylation with dimethyl sulfate, V gave pinostrobin methyl ether. The UV, IR, and NMR spectral data and the mp of the isolated original compound agreed well with those of the pinostrobin which was isolated from *Kaempferria pandurata* (ZINGIBERACEAE).[10] It is well known that pinostrobin is widely distributed in Pinaceae.[11-14]

Alipinetin (5-Methoxy-7-hydroxy-flavanone) (VI). The extensive chromatography of the phenolic portion afforded a flavanone as white needles (M^+ 270, mp 224.5—225.5°C, R_f=0.65 (benzene-methanol-acetic acid 5 : 1 : 1 vol%), negative coloration with ferric chloride and with magnesium-hydrochloric acid). The NMR spectrum of VI in trifluoroacetic acid indicates signals quite similar to those of pinostrobin. The effect upon the mass spectrum of VI is also closely analogous to that of pinostrobin and 5,7-dimethoxyflavanone. When VI was methylated, it gave pinostrobin methyl ether as fine needles. The UV and IR spectra and the mp of the isolated flavanone (VI) showed good agreement with those of alpinetin.[10]

Mongkolsuk and Dean[10] found that pinostrobin and alpinetin both occurred in *Kaempferria pandurata* (ZINGIBERACEAE) and considered that flavanoids possessing a 5-methoxy group are unusual in plants. There is no record of the coexistence of pinostrobin and alpinetin in Dicotyledoneae.

Cinnamic Acid (VII). A large quantity of colorless needles (mp 134.0—134.5°C) was isolated from the acidic portion of the extract regenerated from the sodium bicarbonate solution. The acidic compound (VII) was converted to methyl cinnamate with diazomethane. The IR and NMR spectra and the mp of the isolated acid (VII) were all completely identical with those of the authentic cinnamic acid.

In addition to the above compounds, paraffin hydrocarbons (*n*-paraffins: *n*-C_{21}—*n*-C_{33}, main component, *n*-C_{27}; anteiso: C_{22}, C_{24} and C_{26}; iso: C_{21}, C_{23} and C_{25}) and triglycerides (acid portions: saturated fatty acids, C_{16}, C_{18}, C_{20}, C_{21}, C_{22}, C_{23} and C_{24}) were tentatively identified by a comparison of the retention time with those of authentic specimens of *n*-paraffins (SE-30 2.5%, 180—300°C and 200°C) and of fatty acid methyl esters (DEGS 10%, 187°C), and by the linear relationship between the logarithm of the retention time and the number of the carbon atom of the paraffins and the fatty acid methyl esters respectively.

Biosynthetic Pathways of the Aromatic Compounds Isolated from *A. firma*. Many natural phenyl-propane derivatives have been found in organisms, and it is now recognized that the fundamental skeleton of phenyl-propane derivatives arises from phenylalanine and its congeners derived from shikimic acid and prephenic acid (these are the key compounds in this series). Birch[15] and Underhill[16] suggested that the skeletons of flavanoids and stilbenes arise from a cinnamic acid unit and three acetate units, and they assumed that, in the biosynthesis of pinosylvin (VIII), one molecule of cinnamic acid condenses with three molecules of acetic acid to give the intermediate (IX), which is then cyclized to pinosylvin carboxylic acid (X) and finally decarboxylated to give pinosylvin (VIII), as is shown in Diagram 2. This hypothesis possesses the further merit that it explains the isolation of two stilbene carboxylic acids (hydrangenic acid (XI) and phyllodulcic acid (XII)) and the coexistence of flavanoids (*e.g.*, pinocembrin (XIII) and its methyl ethers) and stilbenes (*e.g.*, pinosylvin (VIII) and its methyl ethers) in the heartwood of many pines[11] and in the buds of some species of Eucalyptus,[17] and from some of these sources cinnamic acid has been isolated. This hypothesis has been confirmed by the investigation of the biosynthesis of pinosylvin and hydrangenic acid using radioactive acetate and glucose.[18,19] Thus, it

8) A. Goris and H. Canal, *Bull. Soc. Chim. Fr.*, **1936**, 1982.
9) H. Audier, *ibid.*, **1966**, 2892.
10) S. Mongkolsuk and F. M. Dean, *J. Chem. Soc.*, **1964**, 4654.
11) V. B. Mahesh and J. R. Seshadri, *Chem. Abstr.*, **49**, 11273 (1955).
12) H. Erdtman, *ibid.*, **40**, 1309 (1946).
13) M. Sogo and K. Hata, *ibid.*, **48**, 12922 (1954).
14) G. Lindstedt and Misiorny, *Acta Chem. Scand.*, **5**, 121 (1951).
15) A. J. Birch and F. W. Donovan, *Aust. J. Chem.*, **6**, 260 (1953); A. J. Birch, *Fortschr. Chem. Org. Naturst.*, **14**, 189 (1957).
16) E. W. Underhill, J. E. Watkin and A. C. Neish, *Can. J. Biochem. Phys.*, **35**, 219, 229 (1957).
17) W. Hills and M. Hasegawa, *Biochem. J.*, **83**, 503 (1962).
18) G. Billek and H. Kindl, *Monatsh. Chem.*, **92**, 493 (1961).
19) R. K. Ibrahim and G. H. N. Towers, *Can. J. Biochem. Phys.*, **38**, 627 (1960); **40**, 449 (1962).

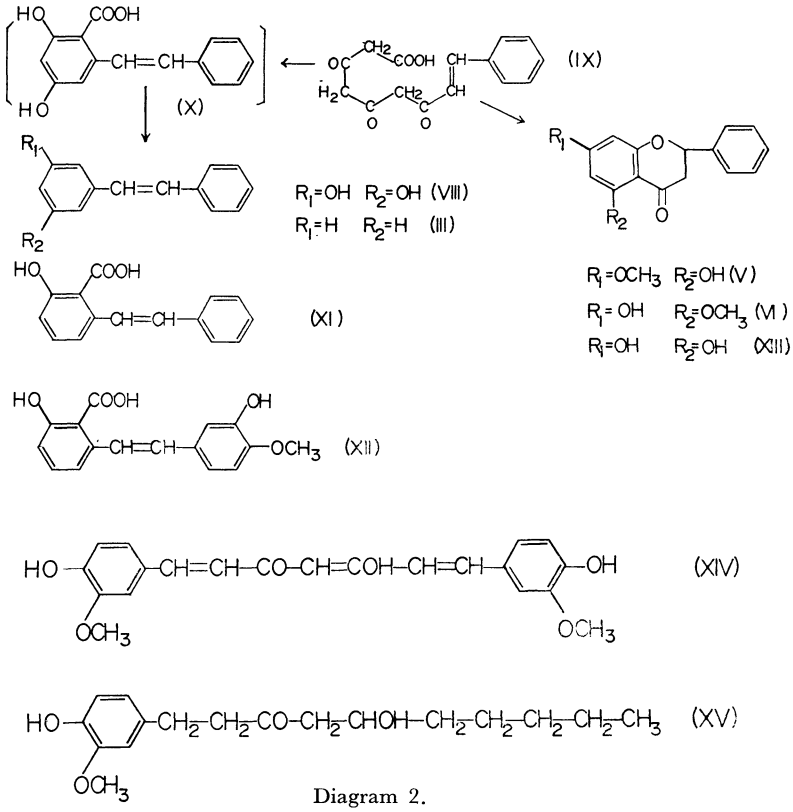


Diagram 2.

can be deduced that the *trans*-stilbene (III), *β*-phenylethyl cinnamate (IV), pinostrobin (V), and alpinetin (VI) isolated from *A. firma* result from a combination of acetic acid and shikimic acid pathways. It is noteworthy that free cinnamic acid is isolated together with pinostrobin, alpinetin, and *trans*-stilbene in this work.

There are many other natural products which also result from the condensation of cinnamic acid and acetic acid units. Thus, yashabushiketol (I) and dihydroyashabushiketol (II) are formed by the candensation of two molecules of cinnamic acid and a molecule of acetic acid. Ketol (I) and ketol (II) possess the same skeleton as the curcumin (XIV) isolated from *Curcuma longa* (ZINGIBERACEAE)[20] and are partially similar to the structure of gingeron (XV) found in *Zingiber officinalis* (ZINGIBERACEAE).[21] Recently, pinostrobin and alpinetin have been isolated from *Kaempferria pandurata* (ZINGIBERACEAE).[10] It is very interesting, from the viewpoint of biosynthesis, that the constituents isolated from *A. firma* (BETULACEAE) are closely analogous to those of ZINGIBERACEAE.

Details regarding the constituents of the cone of *A. firma* and *Alnus multinervis* (Himeyashabushi in Japan) will be reported in a later publication.

Experimental

All the melting points described are uncorrected. The IR spectra were recorded on a Perkin-Elmer model 621 grating Infrared Spectrophotometer in liquid film, in chloroform, in Nujol paste, or as KBr tablets. The NMR spectra were measured on a Varian Asssociates A-60 or Hitachi Perkin-Elmer model R-20 Spectrometer, operating at 60 MHz, and were examined in a 5—10% solution of deuterochloroform, using tetramethylsilane as the internal standard, unless otherwise indicated. The following abbreviations are used for the representation of the NMR data: s=singlet, d=doublet, t=triplet, q=quartet, bs=broad singlet, quin.=quintet, and m=multiplet. The UV spectra were measured on a Shimadzu Recording Spectrophotometer, SV 50 A type, in a 95% ethanol solution. The mass spectra were recorded on a JEOL JMS-01SG Mass Spectrometer under the following conditions: ion accel. volt, 7 kV; ionizing energy, 75 eV; ionizing current, 200 μA; vacuum, 1×10^{-7} Torr; sample temp., 110°C; chamber temp., 250°C; direct sample-introduction system with a vacuum lock. The high-resolution mass spectra were measured on a JEOL JMS-01SP high-resolution mass spectrometer under the following conditions: ionizing energy, 75 eV; direct inlet system; sample temp., 120°C; reference substance, perfluorokerocene. The gas chromatograms were measured on a Hitachi Perkin-Elmer F6-D type Gas chromatograph equipped with a flame-ionization detector. Stainless-steel columns (2 m×2 mm)

20) J. Milobedzka, S. Kostanecki and V. Lampe, *Ber.*, **43**, 2163 (1910); K. R. Srinivasan, *J. Pharm. and Pharmacol.*, **5**, 448 (1953).

21) H. Nomura, *Tohoku Riho*, **7**, 69 (1918).

coated with SE-30 2.5%, PEG 6000 10%, NGS 10% and DEGS 10% on Diasolid L (60—80 mesh) were used. Thin-layer chromatography was carried out with Silica gel G (Merck) under the following conditions: solvent system, benzene - ethyl acetate 19 : 1 vol% unless otherwise stated; chromogenic agent, iodine vapor or mixed acid (conc. H_2SO_4: conc. HNO_3 19 : 1 vol%); developing distance, 15 cm.

Extraction and Isolation Procedure. Ninety-four kg of young shoots of *A. firma* grown in Hiroshima were collected in February and were extracted with redistilled benzene for three weeks. The evaporation of the solvent gave 5 kg of a dark-green-colored slurry. The extract (18.5 g), after the acidic and phenolic constituents has been removed by treatment with 5% sodium bicarbonate and 5% sodium hydroxide solutions, was fractionated into three portions by silica-gel chromatography using *n*-hexane, benzene, and ethyl acetate as eluting agents successively. The extract (4 g) by *n*-hexane was further chromatographed over silica gel using *n*-hexane. Likewise, the extract (7 g) by benzene and the extract (4 g) by ethyl acetate were each further chromatographed using the same solvent as before. The acidic portion (4 g) regenerated from the sodium bicarbonate solution by 5% hydrochloric acid was purified through a silica-gel column using ethyl acetate. The phenolic portion (14 g) regenerated from the sodium hydroxide solution by 5% hydrochloric acid was also chromatographed on silica gel using chloroform - acetone (19 : 1).

Yashabushiketol (I) (1,7-Diphenyl-5-hydroxy-1-hepten-3-one). From the ethyl acetate fraction, a ketol was isolated as colorless needles (150 mg); mp 59.5—60.5°C (from *n*-hexane), $[\alpha]_D^{20}$ +29.0° (*c*, 1.05 in $CHCl_3$), R_f=0.14 (benzene - ethyl acetate 19 : 1 vol%) and R_f=0.52 (the same solvent 3 : 2). This compound was identified as 1,7-diphenyl-5-hydroxy-1-hepten-3-one from the chemical and spectral evidence as has been mentioned in the "Results and Discussion" section.

Catalytic Hydrogenation of Yashabushiketol (I). Yashabushiketol (37 mg) was added to a prereduced PtO_2 catalyst (20 mg) in glacial acetic acid (1.5 m*l*). The solution was then stirred at room temperature, whereupon it absorbed one mole equivalent of hydrogen in 2 hr. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo* to take off the solvent. The resulting oily yellow substance was chromatographed on a silica-gel column using ethyl acetate to give white needles (25 mg); mp 36.0—37.0°C (after crystallization from *n*-hexane), R_f=0.25 (benzene - ethyl acetate 19 : 1). Its IR and NMR spectra agreed with those of dihydroyashabushiketol, to be discussed below.

Acetylation of Yashabushiketol (I). A solution of 20 mg of yashabushiketol in 2 m*l* of anhydrous pyridine was treated with acetic anhydride and then allowed to stand overnight. The mixture was then decomposed with ice water, and the solvent was removed *in vacuo*. The product was chromatographed on a silica-gel column using ethyl acetate to give a yellow oil (12 mg). R_f=0.5; IR spectrum; ν_{max}^{liq} 3080, 3060, 3027, 2930, 1930, 1860, 1790, 1735, 1659, 1625, 1569, 1495, 1455, 1373, 1331, 1239, 1028, 969, 748 and 749 cm^{-1}, NMR spectrum; $\delta_{ppm}^{CDCl_3}$ 5.40 (quin. J=7 Hz, 1H, $-CH_2-C\underline{H}OAc-CH_2-$), 2.04 (s, 3H, $C\underline{H}_3CO-$).

Dihydroyashabushiketol (II) (1,7-Diphenyl-5-hydroxy-3-heptanone). The repeated chromatography of the substance from ethyl acetate fractions gave colorless needles (80 mg) (mp 36.0—37.0°C (after crystallization from *n*-hexane), R_f=0.25) which were identified as 1,7-diphenyl-5-hydroxy-3-heptanone on the basis of the results of chemical and spectrometric treatment. Found: C, 83.80; H, 7.68%. Calcd for $C_{19}H_{22}O_2$: C, 83.69; H, 7.80%.

***trans*-Stilbene (III).** The crude *trans*-stilbene obtained from the *n*-hexane extract was recrystallized from ether to yield colorless leaflet crystals (20 mg); mp 120.0—121.0°C (lit,[7] 120.7°C), R_f=0.75, IR spectrum; ν_{max}^{EtOH} 3016, 1590, 1491, 1451, 755 and 690 cm^{-1} (monosubstituted benzene-ring), 959 (*trans* double bond); UV spectrum: λ_{max}^{EtOH} 228.5 mμ (log ε, 4.22), 236 (4.03), 295 (4.46), 307 (4.42) and 320 (4.23), NMR spectrum: $\delta_{ppm}^{CDCl_3}$ 7.41 (symm. m, 10H, two monosubstituted benzene-ring protons) and 7.18 (s, 2H, *trans* ethylenic protons), mass spectrum; M^+ 180 (base peak), *m/e* 179, 178, 165 (M−15, *m**=151.2), 152, 91, 89, 77, 63, 51 and 39. These data agreed with those of the authentic *trans*-stilbene. The mp was not depressed on admixture with an authentic sample.

β-Phenylethyl Cinnamate (IV). From the substance of the benzene extract, a colorless, viscous oil was obtained. This oil was then chromatographed on silica gel, using benzene, to give white crystals; repeated crystallization from *n*-hexane yielded colorless needles (1.067 g). Mp 56.5—57.0°C, R_f=0.50, UV spectrum; λ_{max}^{EtOH} 216.5 (log ε, 4.29), 223 (4.13), and 277 (4.34), IR spectrum; ν_{max}^{Nujol} 3060, 3010, 1575, 1500, 1470, 760, 695 (monosubstituted benzene-ring), 1710, 1285, 1170 (α,β-unsaturated ester), 1640, 980 (*trans* double bond), 1330, 1315, 1210, 1070, 900, 865, 750, 730 and 680 cm^{-1}, NMR spectrum; $\delta_{ppm}^{CDCl_3}$ 7.43 (bs, 5H monosubstituted benzene-ring protons), 7.30 (s, 5H, monosubstituted benzene-ring protons), 7.71 (d, J=16.2 Hz, 1H, $Ar-C\underline{H}=CH-$), 6.41 (d, J=16.2 Hz, 1H, $-CH=C\underline{H}-CO-$), 4.45 (t, J=7 Hz, 2H, $O-C\underline{H}_2-CH_2-Ar$), and 3.03 (t, J=7 Hz, 2H, $-CH_2-C\underline{H}_2-Ar$), mass spectrum; M^+ 252, *m/e* 104 (base peak, $Ar-CH=CH_2$),$^+$ 148, 147, 131 $(Ar-CH=CH-CO)^+$, 105 $(Ar-CH_2-CH_2)^+$, 103 $(Ar-CH=CH)^+$, 91 $(C_7H_7)^+$, 77, 65, 51 and 39, GLC Rt: 36.2 min. (NGS 10%, 212°C). All these data were completely identical with those of authentic β-phenylethyl cinnamate.

Alkaline Hydrolysis of β-Phenylethyl Cinnamate (IV). A solution of β-phenylethyl cinnamate (80 mg) in methanol was hydrolyzed with an alcoholic potassium hydroxide (about 1N). Colorless oil (10 mg) was obtained from the ether extract of the reaction mixture. The IR and GLC Rt were identical with those of authentic β-phenylethyl alcohol. White crystals (30 mg) were obtained by acidifing the potassium salt of the saponified substance with dilute hydrochloric acid. The IR and NMR spectra and the mp (134.0—135.0°C) showed the crystalline substance to be cinnamic acid.

Pinostrobin (5-Hydroxy-7-methoxyflavanone) (V). From the substance of the benzene extract, pale yellow crystals (100 mg) were obtained. Crystallization from *n*-hexane afforded colorless plates; mp 99.0—100.0°C (pinostrobin, 100°C,[10] 112—113°C[11] and 90—91°C[12]), R_f=0.48. The compound showed positive coloration with ferric chloride and with magnesium - hydrochloric acid. UV spectrum; λ_{max}^{EtOH} 215 mμ (log ε, 4.38), 228.5 (4.20), 290 (4.22) and 330 (3.50), IR spectrum: ν_{max}^{KBr} 3032, 2964, 2916, 2834, 1633, 1570, 1464, 1444, 1381, 1353, 1342, 1295, 1256, 1208, 1199,

1155, 1090, 1060, 992, 941, 890, 834, 794, 765 and 745 cm^{-1}, NMR spectrum: $\delta_{ppm}^{CDCl_3}$ 12.06 (s, 1H), 7.47 (s, 5H), 6.11 (s, 2H), 5.44 (q, J_{AX}=11 Hz, J_{BX}=5 Hz, 1H), 3.81(s, 3H), 2.99 (d, J_{AX}=11 Hz, 1H) and 2.91 (d, J_{BX}=5 Hz, 1H), mass spectrum; M^+ 270 (base peak), *m/e* 269, 193, 167, 166, 159, 138, 123, 114, 110, 104, 103, 95, 91, 78, 77, 69, 51 and 39.

Pinostrobin Monoacetate. Pale yellow crystals (15 mg), mp 142.0—144.0°C, were prepared by treating a solution of 29 mg of pinostrobin in 1 m*l* of anhydrous pyridine with 3 m*l* of acetic anhydride. R_f=0.55, IR spectrum; ν_{max}^{Nujol} 1740, 1255, 1050 (acetate band), 1655, 1600, 1550, 1430, 1325, 1310, 1260, 1240, 1190, 1145, 1020, 890, 865, 835, 760 and 695 cm^{-1}.

Methylation of Pinostrobin (V). A mixture of pinostrobin (30 mg), dimethyl sulfate (5 m*l*), pottasium carbonate (150 mg), and dry acetone (10 m*l*) was refluxed on a water bath for 48 hr to give pinostrobin methyl ether as white needles. Mp 142.0—143.0°C (pinostrobin methyl ether, 144°C[10]), R_f=0.58, IR spectrum; $\nu_{max}^{CHCl_3}$ 3025, 2955, 2950, 1979, 1895, 1670, 1600, 1570, 1460, 1430, 1370, 1340, 1320, 1270, 1209, 1180, 1110, 1070, 1040, 1030, 965, 940, 890, 860, 820, 800, 700 and 660 cm^{-1}.

Alpinetin (5-Methoxy-7-hydroxyflavanone) (VI). The phenolic portion (14 g) of the extract regenerated from the sodium hydroxide solution by hydrochloric acid was fractionated through a silica-gel column with chloroform-acetone (19 : 1 vol%); a small quantity of white crystals was thus obtained. Recrystallization from methanol gave white needles (30 mg) which showed the following properties: mp 224.5—225.0°C (alpinetin, 220°C[10] 223°C[22]), R_f=0.65 (benzene - methanol - acetic acid 5 : 1 : 1). Ferric chloride and magnesium - hydrochloric acid tests were negative. UV spectrum; λ_{max}^{EtOH} 231 (log ε, 4.08), 237 (3.98), 285 (4.21) and 310 (3.75), $\lambda_{max}^{EtOH+NaOAc}$ 230 mμ (log ε, 4.06), 237 (3.87), 252.5 (3.57), 287 (4.04) and 323 (4.15), IR spectrum; ν_{max}^{KBr} 3520, 3300, 3030, 2935, 1633, 1611, 1575, 1479, 1458, 1435, 1358, 1302, 1276, 1249, 1206, 1176, 1101, 1068, 1056, 980, 833 and 766 cm^{-1}, NMR spectrum; $\delta_{ppm}^{CF_3COOH}$ 7.51 (s, 5H), 6.47 (s, 2H), 5.71 (q, J_{AX}=11Hz, J_{BX}=6 Hz, 1H), 4.30 (s, 3H), 3.49 (d, J_{AX}=11 Hz, 1H) and 3.40 (d, J_{BX}=6 Hz, 1H), mass spectrum; M^+ 270, *m/e* 166 (base peak), 193, 167, 138, 123, 114, 104, 103, 95, 78, 77, 69, 51, 39, 18 and 17.

Methylation of Alpinetin (VI). By the same method as in methylation of pinostrobin, pinostrobin methyl ether was obtained as white crystals (14 mg). Mp 144.0—145.0°C (lit,[10] 144°C), R_f=0.58. Its IR spectrum agreed with that of pinostrobin methyl ether.

Cinnamic Acid (VII). The acidic portion (4 g) of the extract regenerated from the sodium bicarbonate solution by dilute hydrochloric acid was purified through silica-gel chromatography to give white needles (1.5 g); mp 134.0—134.5°C (from ether). The IR and NMR spectra were completely identical with those of an authentic specimen.

Paraffin Hydrocarbon. The fractions eluted with *n*-hexane yielded paraffin hydrocarbons (0.8 g). Mp 51.0—54.0°C, R_f=0.78, IR spectrum; $\nu_{max}^{CHCl_3}$ 2990, 2950, 1475, 1385, 1190, 1160, 740 and 735 cm^{-1}. *n*-Paraffins (C_{21}—C_{33}), anteiso paraffins (C_{22}, C_{24} and C_{26}) and iso paraffins (C_{21}, C_{23} and C_{25}) were tentatively identified by a comparison with the retention times of the authentic *n*-paraffins.

Triglyceride. A white crystalline mass (1.76 g) was obtained from the *n*-hexane extract. When it was recrystallized from chloroform, white crystals were obtained. Mp 76.0—77.0°C, R_f=0.6, IR spectrum; ν_{max}^{KBr} 2960, 2930, 2850, 1740, 1465, 1420, 1378, 1210, 1190, 1180, 920, 725 and 718 cm^{-1}, NMR spectrum; $\delta_{max}^{CDCl_3}$ 4.08 (m), 2.25 (m), 1.28 (s) and 0.90 (m). The alkaline hydrolysis (5% NaOH-methanol) of this mass (1.0 g) gave fatty acid mixtures (saturated, C_{16}, C_{18}, H_{20}, C_{21}, C_{22}, C_{23}, C_{24} and C_{25}) which were confirmed as methyl esters by gas chromatography (DEGS 10%, 187°C).

The author wish to express his hearty gratitude to Professor Tamon Matsuura, Professor Shuichi Hayashi and Assistant Professor Takayuki Suga of Hiroshima University for their guidance and encouragement. The author is also indebted to Mr. Fumihide Genjida for his help in this investigation. Grateful acknowledgement is also made to Professor Shô Itô of Tohoku University and Dr. Hiroshi Ishii of Shionogi Research Laboratory, Shionogi & Co., Ltd. for the measurement of NMR spectra. The author also thanks for Japan Electron Optics Co., Ltd., for high-resolution mass spectral determination.

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