Chemical Constituents of the Male Flower of *Alnus pendula* (BETULACEAE)

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(Received January 26, 1972)

The structure of alnustone (**1**) isolated from the male flower of *Alnus pendula* Matsum. has been established to be 1,7-diphenyl-1,3-heptadien-5-one. Previously-known compounds, β-phenylethyl cinnamate (**6**), cinnamaldehyde, benzylacetone, β-phenylethyl alcohol, pinocembrin (**7**), pinostrobin (**8**), alpinetin (**9**), galangin (**11**), pinosylvin (**13**), and its monomethyl ether (**14**), eugenol, chavicol, cinnamic acid, β-phenylpropionic acid, and benzoic acid, were identified along with the above new ketone.

Alnus pendula Matsum. (*A. multinervis* Call.)[1] belongs to Betulaceae and can be used as a water-erosion-control plant. Previous studies[2-6] of the chemical constituents of the male flower of *Alnus sieboldiana* (Betulaceae) have shown the presence of two new ketols, yashabushiketol (**4**) and dihydroyashabushiketol (**5**), with a biosynthetically interesting skeleton, four new flavonoids, together with twenty-five aromatic compounds, and small amounts of aliphatic and isoprenoid compounds. The present paper will describe the isolation and structure elucidation of a new ketone, named alnustone (**1**),[7] with the same Ph–C_7–Ph skeleton as that of the ketols (**4**) and (**5**) and the identification of fifteen aromatic compounds from the male flower of *A. pendula*. From the viewpoint of biosynthesis and chemotaxonomy, the chemical constituents of the male flower of *A. pendula* will also be compared with those of *A. sieboldiana*.

Results and Discussion

The benzene extraction of a viscous material secreted on the fresh male flower (catkin) of *A. pendula* Matsum. gave a brown viscous oil. After the removal of the acidic and phenolic constituents, a neutral fraction was separated into three fractions by silica gel column chromatography, using *n*-hexane, benzene, and ethyl acetate successively as eluents.

Alnustone (**1**). The chromatography of the benzene-elution fraction over silica gel yielded alnustone (**1**) in fairly considerable amounts as pale yellow needles (mp 61.0—62.5°C). Its formula, $C_{19}H_{18}O$, was based on the high-resolution mass spectral analysis of the molecular ion. The spectral properties indicated that alnustone possessed a *trans*, *trans*-dienone group (ν_{max} 1665 and 990 cm^{-1}; λ_{max} 325 nm (log ε 4.40) and two monosubstituted benzene-rings (ν_{max} 750 and 700 cm^{-1}; δ 7.11 (s, 5H) and 7.20 ppm (m, 5H)). The base peak (*m/e* 157) and the signals at δ 6.11 (d, 1H, –CH=CH–CO–), 6.77 (m, 2H, =CH–CH=), and 7.18 ppm (m, 1H, overlapped with the signals of benzene-ring protons, Ph–CH=CH–) indicated the presence of the Ph–CH=CH–CH=CH–CO– group. The presence of the Ph–CH_2–CH_2–CO– group was confirmed by the NMR signals at δ 2.82 ppm (m, 4H). The hydrogenation of **1** over the platinum oxide catalyst readily gave the tetrahydroderivative (**2**), $C_{19}H_{22}O$, which was established on the bases of the disappearance of absorption bands due to the *trans*, *trans*-dienone moiety and the newly-appearing absorption bands of an isolated carbonyl group. On the other hand, the reduction of **1** by sodium borohydride afforded a secondary alcohol (**3**) with a conjugated diene group adjacent to the benzene ring, judging from its spectral data. Thus, alnustone has been confirmed to be *trans*, *trans*-1,7-diphenyl-1,3-heptadien-5-one (**1**).

Known Aromatic Compounds. *a) Neutral Components*: The isolation of β-phenylethyl cinnamate (**6**), cinnamaldehyde, benzylacetone, and β-phenylethyl alcohol was performed by a combination of the silica gel column chromatography and the preparative thin-layer chromatography of the neutral fractions. All the compounds isolated were identified by a direct comparison of their physical and spectral data with those of the authentic samples or with those reported in our previous papers.[2-6]

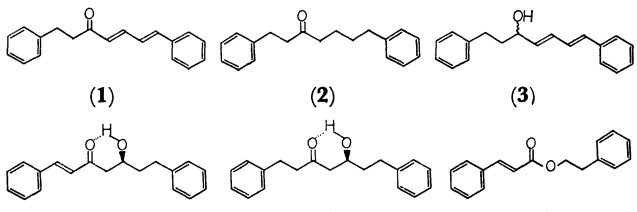

Fig. 1.

b) Phenolic and Acidic Components: Four flavonoids, pinocembrin (**7**), pinostrobin (**8**), alpinetin (**9**), and galangin (**11**), two stilbenes, pinosylvin (**13**) and pinosylvin monomethyl ether (**14**), and two phenols, eugenol and chavicol, were isolated from the phenolic fraction and subsequently identified by the above method. As acidic components, cinnamic acid, β-phenylpropionic acid, and benzoic acid were identified by a direct comparison of their retention times in gas chromatography and of the spectra of the methyl esters of authentic specimens.

A Comparison between the Constituents of the Male Flower of A. pendula and A. sieboldiana. The first object of this study was to compare the occurrence and distribution of the Ph–C_3 compounds in *A. pendula* with those

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2) Y. Asakawa, F. Genjida, S. Hayashi, and T. Matsuura, *Tetrahedron Lett.*, **1969**, 3235.
3) Y. Asakawa, This Bulletin, **43**, 575 (1970).
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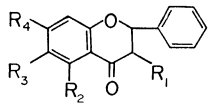
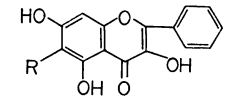
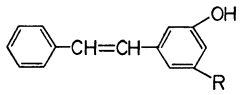
7) We have reported a part of the structural elucidation of alnustone (**1**) in the form of a preliminary communication on *Chem. Ind.* (London), **1971**, 766.



(**7**) $R_1=R_3=H$, $R_2=R_4=OH$
(**8**) $R_1=R_3=H$, $R_2=OH$, $R_4=OMe$
(**9**) $R_1=R_3=H$, $R_2=OMe$, $R_4=OH$
(**10**) $R_1=R_2=R_4=OH$, $R_3=OMe$



(**11**) R=H
(**12**) R=OMe



(**13**) R=OH
(**14**) R=OMe

Fig. 2.

of the compounds in *A. sieboldiana* from the points of view of biosynthesis and chemotaxonomy. β-Phenylethyl cinnamate (**6**) was most abundant in the male flower of both plants. Alnustone (**1**) was found in *A. pendula*, but not in *A. sieboldiana*. On the other hand, yashabushiketol (**4**) and dihydroyashabushiketol (**5**) appeared only in *A. sieboldiana*. These facts are very interesting with respect to the biosynthesis of these three components. The co-occurrence of flavonoids and stilbenes was found in both plants. Four flavonoids with the $C_{(6)}$–OMe group, such as alnustinol (**10**) and alnusin (**12**), have been found in *A. sieboldiana*, in contrast with the case of *A. pendula*. One of the most significant discoveries of the present survey is that the great majority of components isolated from *A. pendula* are composed of simple aromatic compounds with one or two Ph–C_3 skeletons, in the same manner as in *A. sieboldiana*, with the exception of eugenol and chavicol. In addition to the above aromatic constituents, small amounts of *n*-paraffins, α-olefins, triterpenes (taraxerone, β-amyrenone, and a triterpenic acid), unsaturated aliphatic ketones, and fatty acids are commonly present in both plants. Thus, it is clear that the two plants belong to closely related species from the viewpoint of chemotaxonomy.

Experimental

All the melting points are uncorrected. The UV spectra were measured by means of a Japan Spectroscopic Co., Ltd., ORD/UV-5 Spectropolarimeter in ethanol. The mass spectra were recorded on a Hitachi, RMS-4, Mass Spectrometer. The high-resolution mass spectra were obtained by means of a Hitachi, RMU-TL, Mass Spectrometer. The NMR spectra (100 MHz) were recorded on a JEOL, JNM-4H-100, High-Resolution Spectrometer in a carbon tetrachloride solution, using tetramethylsilane as the internal standard. The measurements of the IR and NMR (60 MHz) spectra and the glc were performed in the same manner as was reported in our preceding paper.[4]

Extraction and Isolation. Male flowers (6.59 kg) of *A. pendula* grown in the suburbs of Hiroshima were collected in March in 1970 and were, after having been minched mechanically, extracted with benzene over a two-month period. The evaporation of the solvent gave a brown viscous oil (3.6%). This viscous oil was successively extracted with a 5% sodium bicarbonate solution (Acid), a 5% sodium carbonate solution (Phenol I), a 0.4% sodium hydroxide solution (Phenol II), and a 5% sodium hydroxide solution (Phenol III); the subsequent acidification of each fraction with 5% hydrochloric acid gave an acidic fraction (2.3 g) and three phenolic fractions, Phenol I (2.5 g), Phenol II (38.2 g), and Phenol III (7.7 g). After the removal of the acidic and phenolic constituents, the neutral fraction (184 g) was obtained. A part (13 g) of it was fractionated into two fractions by silica gel column chromatography using *n*-hexane (Fraction 1) and benzene (Fraction 2). The Fraction 2 was further subjected to silica gel column chromatography to separate it into three fractions (i), (ii), and (iii), using benzene as the eluting agent.

*Alnustone (**1**).* From the fraction (i), a conjugated ketone (**1**) was isolated as pale yellow needles (236 mg): mp 61.0—62.5°C (from ethanol); *m/e* 262.1360 (calcd for $C_{19}H_{18}O$: 262.1357), 171 (Ph–CH=CH–CH=CH–CO–CH_2)$^+$, 157 (base, Ph–CH=CH–CH=CH–CO)$^+$, 129 (Ph–CH=CH–CH=CH)$^+$, 105 (Ph–CH_2–CH_2)$^+$, 103 (Ph–CH=CH)$^+$, 91 (C_7H_7)$^+$, 77 (C_6H_5)$^+$. These fragment ions were identified on the basis of a high-resolution mass-spectral analysis.

Found: C, 86.60; H, 7.19%. Calcd for $C_{19}H_{18}O$: C, 86.98; H, 6.91%.

*The Catalytic Hydrogenation of Alnustone (**1**).* Alnustone (**1**) (42 mg) was hydrogenated over a prereduced PtO_2 catalyst (10 mg) in ethanol for 1 hr under ambient conditions. After the catalyst had then been filtered off, the removal of the solvent from the filtrate gave the tetrahydroderivative (**2**) (34 mg): λ_{max}^{EtOH} 288 nm (log ε 1.99); $\nu_{max}^{liq.}$ 1710 cm^{-1}; δ ($CDCl_3$) 1.4—1.9 (m, 4H, Ph–CH_2–C<u>H</u>$_2$–C<u>H</u>$_2$–CH_2–CO–), 2.2—3.0 (m, 8H, 2×Ph–C<u>H</u>$_2$– and –C<u>H</u>$_2$–CO–C<u>H</u>$_2$–), 7.21 ppm (s, 10H); *m/e* 266 (M$^+$), 175 (Ph–CH_2–CH_2–CH_2–CH_2–CO–CH_2)$^+$ or (Ph–CH_2–CH_2–CO–CH_2–CH_2–CH_2)$^+$, 161 (Ph–CH_2–CH_2–CH_2–CH_2–CO)$^+$, 133 (Ph–CH_2–CH_2–CO)$^+$, 105 (Ph–CH_2–CH_2)$^+$, 91 (base, C_7H_7)$^+$, 77 (C_6H_5)$^+$.

*The Reduction of Alnustone (**1**) with Sodium Borohydride.* Into a solution of **1** (140 mg) in methanol (20 m*l*), we stirred sodium borohydride (60 mg) dissolved in methanol (10 m*l*) at 0°C. The mixture was then stirred for 1 hr. The reaction mixture was acidified with 1N hydrochloric acid, and then the solvent was removed *in vacuo*. The product, after water (10 m*l*) had been added, was extracted with ether. The removal of the solvent from the dried extract gave a secondary alcohol (**3**) (134 mg): λ_{max}^{EtOH} 222 (log ε 4.06), 228 (4.06), 235 (3.94), 288 nm (4.50); $\nu_{max}^{liq.}$ 3400, 1100 (OH), 1640, 990 (*trans*-CH=CH–CH=CH–), 750, 690 cm^{-1} (monosubst. benzene-ring); δ ($CDCl_3$) *ca.* 1.9 (m, 2H, –CH(OH)–C<u>H</u>$_2$–CH_2–Ph), 2.08 (bs, 1H, O<u>H</u>, disappeared upon the addition of D_2O), *ca.* 2.7 (m, 2H, Ph–C<u>H</u>$_2$–), 4.10 (q, J=7 Hz, 1H, =CH–C<u>H</u>(OH)–CH_2–), 5.80 (dd, J=15 Hz and 7 Hz, 1H, –CH=C<u>H</u>–CH(OH)–), 6.1—6.8 (m, 3H, Ph–C<u>H</u>=C<u>H</u>–C<u>H</u>=), 7.24 (s, 5H, C<u>$_6$H$_5$</u>–CH_2–), 7.33 ppm (m, 5H, C<u>$_6$H$_5$</u>–CH=); *m/e* 264 (M$^+$), 246 (M−H_2O), 173 (Ph–CH=CH–CH=CH–CHOH–CH_2)$^+$, 159 (Ph–CH=CH–CH=CH–CHOH)$^+$, 155 (Ph–CH=CH–CH=CH–CH=CH)$^+$, 129 (Ph–CH=CH–CH=CH)$^+$, 117 (Ph–CH_2–CH=CH)$^+$, 105 (Ph–CH_2–CH_2)$^+$, 91 (C_7H_7)$^+$, 77 (C_6H_5)$^+$, 65 (C_5H_5)$^+$.

n-Paraffins, α-Olefins, Unsaturated Aliphatic Ketones, and Triterpenes (Taraxerone and β-Amyrenone). The Fraction 1, when eluted with *n*-hexane, gave a mixture of *n*-paraffins and α-olefins, (1.5 g). The *n*-paraffins (C_{21}–C_{31}: C_{27} main) and α-olefins, (C_{23}–C_{29}) were identified by a comparison

with the retention times in gas chromatography of authentic samples and by the hydrogenation of the mixture. After the isolation of alnustone (**1**) from the fraction (i), the filtrate which showed three spots on tlc was purified by preparative thin-layer chromatography to give a mixture of unsaturated aliphatic ketones (10 mg), mp 71—74°C, β-amyrenone (44 mg), and taraxerone (127 mg).

β-Amyrenone: mp 178—179°C (lit,[8] mp 178—179°C); ν_{max}^{Nujol} 1710 cm^{-1}; *m/e* 424 (M^+) 409, 218 (base), 203, 189. All the spectral and physical data of this compound agreed with those of β-amyrenone derived from β-amyrin.

Taraxerone: mp 233—235°C (lit,[9] mp 237—238°C); ν_{max}^{Nujol} 1705 cm^{-1}; *m/e* 424 (M^+), 409, 300, 285, 204 (base), 218, 189, 133.[9]

*Pinostrobin (**8**), β-Phenylethyl Cinnamate (**6**), β-Phenylethyl Alcohol, Benzylacetone, and Cinnamaldehyde.* The fraction (ii) afforded a colorless viscous oil, which was then further chromatographed on a silica gel column, using a mixture of *n*-hexane and ethyl acetate, to give β-phenylethyl cinnamate (2.170 g), mp 51—52°C, pinostrobin (96 mg), mp 91—92.5°C, and β-phenylethyl alcohol (220 mg). The fraction (iii) gave a pale yellow oil (30 mg) which showed two spots on tlc and two peaks (1: 1) on the gas chromatogram (R_t=4.0 and 7.1, 10% PEG 6000 on Diasolid L (60—80 mesh) at 180°C). The chromatographic separation of the oil by tlc gave cinnamaldehyde (R_t=7.1) and benzylacetone (R_t=4.0). All of the spectral data of the compounds were identical with those of the authentic samples.

*Pinocembrin (**7**) and Galangin (**11**).* The Phenol I (2.5 g) was chromatographed on a silica gel column, using ethyl acetate as the eluting agent, to give pinocembrin (340 mg), mp 201—202.5°C (lit,[6] mp 200—201°C) and galangin (460 mg), mp 220—222°C (lit,[10] 214—215°C). The 3,7-dimethylether derivative: mp 145—147°C (lit,[11] 145—146°C). The spectral data of these compounds coincided completely with those of the known specimens.

*Pinosylvin (**13**), Pinosylvin Monomethyl Ether (**14**), Alpinetin (**9**), Eugenol, Chavicol, a Triterpenic Acid, and Benzoic Acid.* The chromatography of the Phenol II (15.5 g) on a silica gel column using a mixture of *n*-hexane and ethyl acetate gave pinosylvin (250 mg), mp 158—159°C (lit,[6] 156—158°C), pinosylvin monomethyl ether (3.5 g), mp 122—122.5°C (lit,[6] 119—120°C), alpinetin (1.18 g), mp 224—225.5°C (lit,[4] mp 224.5—225.0°C), eugenol (140 mg), n_D^{25} 1.5410, chavicol (60 mg), n_D^{25} 1.5405, benzoic acid (1.93 g), mp and mixed mp 122—123°C, and an unidentified triterpenic acid (50 mg), mp 195—196°C, M^+ 470. The identities were established by a comparison of their spectral data.

Pinosylvin monomethyl ether (1.54 g) and benzoic acid (840 mg) were also isolated from the Phenol III (7.7 g).

β-Phenylpropionic Acid, Cinnamic Acid, and Fatty Acids. The acidic fraction (2.3 g) was methylated with diazomethane. The methyl ester was then chromatographed on silica gel to give methyl cinnamate (50 mg), minute amounts of methyl β-phenylpropionate and methyl benzoate, and a trace amount of a mixture of methyl myristate, methyl palmitate, and methyl stearate. These compounds were identified by a direct comparison of their retention times in glc (10% SE-30 and 10% DEGS on Diasolid L (60—80 mesh) at 100°C) and their IR spectra with those of authentic samples.

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