Studies on the Chemical Constituents of *Xanthoxylum nitidum* (ROXB.) D. C. (*Fagara nitida* ROXB.). III.[1] The Chemical Constituents of the Wood

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The chemical constituents of the wood of *Xanthoxylum nitidum* (ROXB.) D. C. (*Fagara nitida* ROXB.) were examined. Two phenylpropanoids, methyl nitinoate (2) and dihydrocuspidiol (3), and a benzodioxane type lignan, nitidanin (4), were newly isolated. The structures of the phenylpropanoids were chemically determined. In addition, the application of a selective insensitive nuclei enhanced by polarization transfer selective (INEPT) technique in the NMR spectrum to the new lignan allowed us to deduce the structure.

Key words *Xanthoxylum nitidum* D. C. (*Fagara nitida*); chemical constituent; phenylpropanoid; benzodioxane lignan; selective insensitive nuclei enhanced by polarization transfer technique

Xanthoxylum nitidum (ROXB.) D. C. (*Fagara nitida* ROXB.) is a plant used as a folk medicine in tropical and subtropical areas, and from the bark of this plant nitidine (**1**),[2] an antitumor-active benzo[*c*]phenanthridine alkaloid,[3] has been isolated. Recent reexamination[4] of the methanol extract of the bark indicated that **1** was a strong inhibitor of DNA topoisomerase I. Independently we have reported the isolation of its chemical constituents from the alkaloidal part of the bark[5] and the separation of the components of the wood using membrane filtration.[1] As a continuing study of this plant we examined the chemical constituents of the wood by a liquid–liquid partition method. In this report we describe the isolation of the chemical constituents, including two new phenylpropanoids and a new benzodioxane type lignan; we also describe the structural elucidation of the new phenylpropanoids by chemical means and of the new lignan by application of a selective insensitive nuclei enhanced by polarization transfer (INEPT) technique[6] in the NMR spectrum.

Results and Discussion

1. The Chemical Constituents of the Wood The wood of this plant was extracted with hot methanol. The methanol extract was divided into three parts, non-phenolic (fr. A) and phenolic (fr. B) alkaloidal fractions and a non-alkaloidal one (fr. C) by the liquid–liquid partition method. Fraction C was further divided into four fractions by the Sohxlet apparatus[5] (Chart 1). Each fraction was purified by column chromatography and/or preparative TLC (p-TLC).

From fr. A, two new phenylpropanoids designated as methyl nitinoate (**2**) and dihydrocuspidiol (**3**) were isolated together with seventeen known components [six quinoline alkaloids: skimmianine,[7] γ-fagarine,[7] 4-methoxy-1-methyl-2-quinolone,[8] edulutine,[9] ribalinine,[10] and isoplatydesmine[10]; four benzo[*c*]phenanthridine alkaloids: nitidine (**1**),[7] chelerythrine,[11] oxynitidine,[7] and isoarnottianamide[7]; four phenylpropanoids: wutaiensal,[12] methyl 2,3,4-trimethoxycinnamate,[13] 3,4,5-trimethoxycinnamaldehyde,[14] and 3,4,5-trimethoxycinnamylalcohol[15]; two

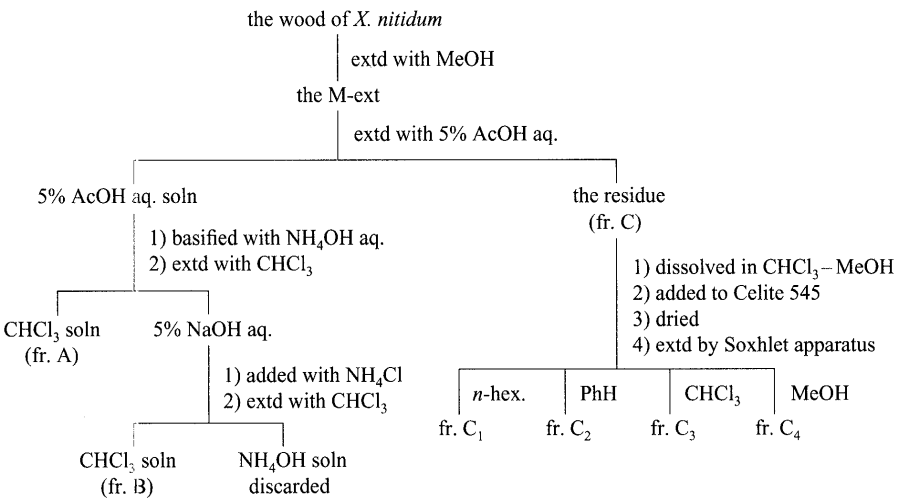

Chart 1

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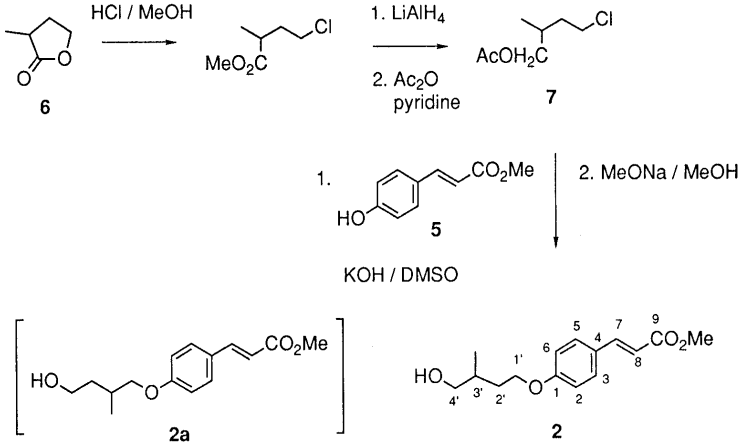
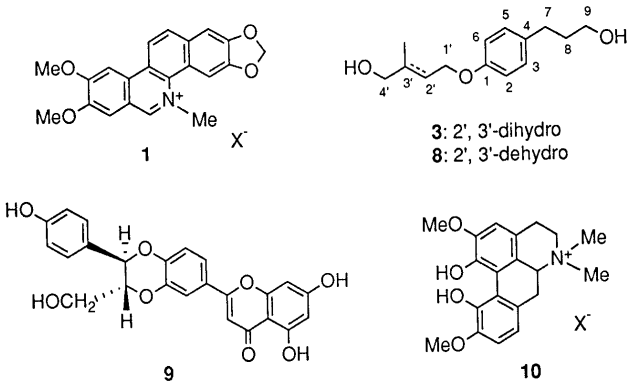


Chart 2



coumarins: 6,7,8-trimethoxycoumarin[8] and aesculetin dimethyl ether,[7] and one aporphine alkaloid: liriodenine[8]].

Additionally, a new lignan, nitidanin (**4**), was isolated from fr. B, together with a known phenolic lignan, syringaresinol.[8]

No isolation of any additional components from fr. C was observed.[16,17]

2. The Structural Determination of New Components Methyl nitinoate (**2**), $C_{15}H_{20}O_4$ (*m/z*: 264), was isolated as pale yellow needles, mp 55.5 °C. The spectral data (see Experimental) allowed us to depict two possible structures of *p*-hydroxycinnamate with a (hydroxymethyl)butyl side chain such as **2** or **2a**. Biogenetic consideration based on the isolation of other phenylpropanoids[7,8] from the related *Xanthoxylum* species indicates a more plausible structure for it as **2**. The validity of the deduction was demonstrated by its synthesis.

Treatment of a *p*-hydroxycinnamate (**5**) with 4-chloro-2-methylbutyl acetate (**7**), which was prepared from 2-methyl-γ-butyrolactone (**6**) by the action of methanolic hydrogen chloride[18] followed by hydride reduction and then acetylation, in the presence of a base, afforded a desired ester (**2**) in 65% yield after methanolysis (Chart 2). Methyl nitinoate (**2**) was optically active, but the absolute stereochemistry remains unknown.

Dihydrocuspidiol (**3**), $C_{14}H_{22}O_3$ (*m/z*: 238), was obtained as colorless needles, mp 52 °C. This new phenylpropanoid could be reasonably assigned to be a reduced product of cuspidiol (**8**)[7] by the spectral data (see Experimental). Its structure, except the absolute stereochemistry, was finally established to be **3** by the catalytic hydrogenation of **8** on Wilkinson's catalyst.

Table 1. The NMR Data[a] of Nitidanin (**4**) (in $CDCl_3$)

C No.	δ_H	δ_C	COLOC[b] 6 Hz	COLOC[b] 8 Hz
2	4.89 (d, J=8.0 Hz)	76.95	2'-H (3), 2-CH_2 (3)	2'-H (3), 2-CH_2 (3)
3	4.05 (m)	79.24		
3-CH_2	3.55 (dd, J=12.6, 4.4 Hz), 3.75 (dd, J=12.6, 2.5 Hz)	61.40		
4a	—	132.97	6-H (3), 8-H (3)	6-H (3), 8-H(3)
5	—	148.81	5-OMe (3)	5-OMe (3)
5-OMe	3.94 (s)	56.41		
6	6.65 (d, J=1.9 Hz)	102.94		8-H (3), 9-H (3)
7	—	130.15		
8	6.69 (d, J=1.9 Hz)	108.70		6-H (3), 9-H (3)
8a	—	144.67	8-H (2)	
9	6.49 (dd, J=15.9, 1.4 Hz)	130.64	6-H (3)	
10	6.22 (dd, J=15.9, 5.8 Hz)	127.92	11-H (2)	
11	4.24 (dd, J=5.8, 1.4 Hz)	63.09	9-H (3)	
1'	—	127.21	2'-H (2)	2'-H (2)
2'	6.68 (s)	104.79		
3'	—	148.23	2'-H (2), 3'-OMe (3)	2'-H (2), 3'-OMe (3)
3'-OMe	3.90 (s)	56.53		
4'	—	136.16	2'-H (3)	2'-H (3)

a) ^{1}H-NMR (500 MHz) are reported downfield from internal tetramethylsilane (TMS) at 0.00 ppm. ^{13}C-NMR (125 MHz) assignment are related to internal $CDCl_3$ at 77.00 ppm. *b*) The number in parentheses denotes the number of bonds involved in the correlation.

Nitidanin (**4**) was isolated as an optically inactive colorless powder, mp 232 °C, and its molecular formulation was assigned as $C_{21}H_{24}O_8$ (Calcd for 404.1471) on the basis of the peak at *m/z*: 404.1468 in the high resolution FAB-MS (HR-FAB-MS). The presence of a phenolic hydroxy (OH) group in **4** was suggested by the absorption band (3494 cm^{-1}) in the IR and the absorption maxima (224, 272 nm) in the UV spectroscopies. The ^{1}H- and ^{13}C-NMR data are shown in Table 1. In the ^{1}H-NMR spectrum of **4** the appearance of a 6H singlet at δ 3.90 and a 2H singlet at δ 6.68 indicated the presence of a

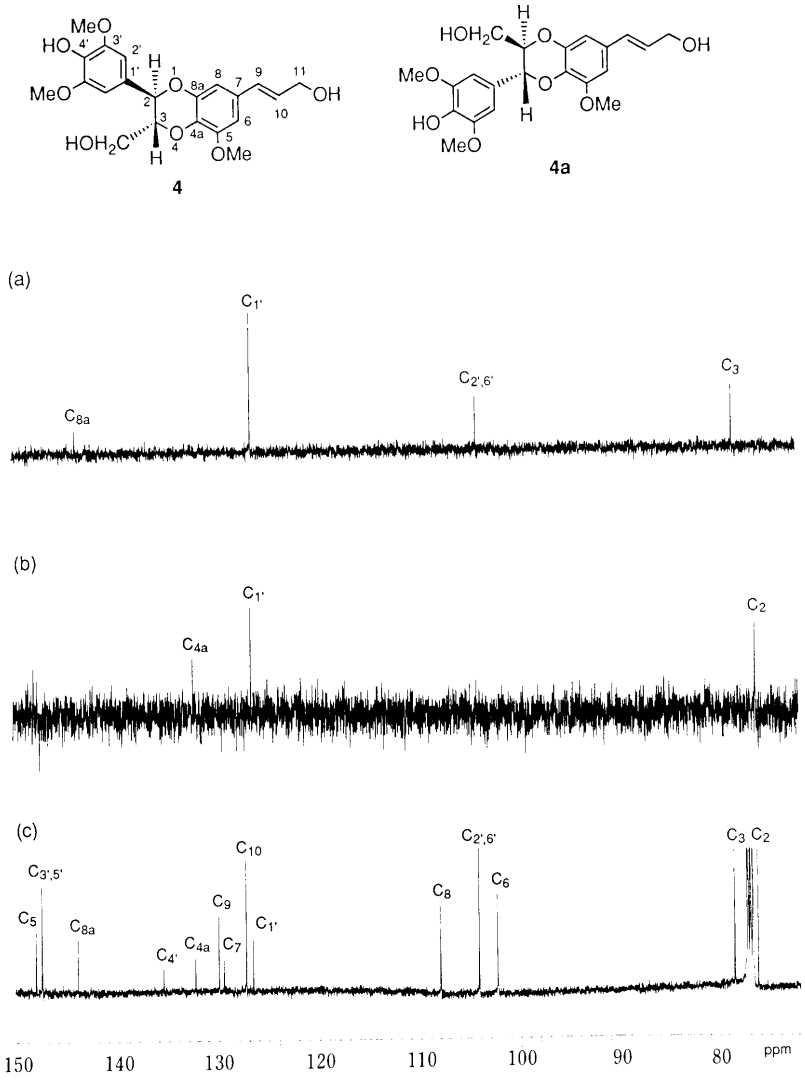


Fig. 1. The Selective INEPT Experiment for Nitidanin (**4**)

(a) Irradiation of C_2-H at δ 4.89. (b) Irradiation of C_3-H at δ 4.05. (c) ^{13}C-NMR spectrum.

symmetrical 4-hydroxy-3,5-dimethoxyphenyl substituent, while a 3H singlet at δ 3.94 and *meta*-coupled doublets ($J=1.9$ Hz) at δ 6.65 and δ 6.69 could be assigned to an unsymmetrical 3-methoxy-4,5-dioxygenated phenyl group. The latter substituent could be expanded to a cinnamyl alcohol unit by the following signal sequence: a 2H doublet ($J=5.8$, 1.4 Hz) at δ 4.24 and two 1H double triplets ($J=15.9$, 5.8 Hz, $J=15.9$, 1.4 Hz) at δ 6.22 and δ 6.49, respectively. A three carbon sequence, CH(O)CH(O)-CH_2O, was deduced by the presence of a 1H multiplet at δ 4.05, a 1H doublet ($J=8.0$ Hz) at δ 4.89, and geminally coupled methylene protons ($J=12.6$ Hz) at δ 3.55 and 3.75. These assignments were confirmed by combination of the ^{13}C-NMR and two-dimensional spectra such as ^{1}H–^{1}H and ^{1}H–^{13}C correlation spectroscopies (COSY) and a correlation between a long range coupling (COLOC) technique. In this stage, two plausible structures (**4** or **4a**) could be drawn for nitidanin. The *trans* stereochemistry between 2-H and 3-H was reasonably deduced by the coupling constant ($J=8.0$ Hz) and then confirmed by signal enhancements of both methine protons on irradiation of a symmetrical aromatic proton at δ 6.68 in the difference nuclear Overhauser effect (NOE) experiment.[19] However, discrimination between **4** and **4a** could not be determined by only these data.

Kinghorn *et al.*[20] reported the structural determination of sinaiticin (**9**), a flavonolignan, by application of a selective INEPT technique in the NMR spectrum. We tried the same technique for the selection of a correct structure for nitidanin (Fig. 1). Irradiation of the methine signal at δ 4.89 (C_2-H) led to signal enhancements of four carbons at δ 79.24 (C_3), δ 104.79 ($C_{2'}$, $C_{6'}$), δ 127.21 ($C_{1'}$), and δ 144.67 (C_{8a}) (Fig. 1a), while three carbon signals at δ 76.95 (C_2), δ 127.21 ($C_{1'}$), and δ 132.97 (C_{4a}) were enhanced on irradiation of the alternative methine signal at δ 4.05 (C_3-H) (Fig. 1b). Thus, we safely assigned the structure of nitidanin as **4**.

Experimental

All melting points were measured on a micro melting-point hot stage (Yanagimoto) and are uncorrected. IR spectra were recorded for KBr pellets on a Hitachi 260-10 or JASCO IR-700 spectrophotometer. NMR spectra were recorded in $CDCl_3$ solution with a JEOL JNM GSX-500α spectrometer, unless otherwise stated, with TMS as an internal reference. Electron impact MS (EI-MS) and HR-FAB-MS were recorded on Hitachi M-60 and JEOL JMX-HX 110A spectrometers, respectively, with a direct inlet system. For column and flash chromatography, Silica gel 60

(70—230 mesh ASTM; Merck) and Silica gel 60 (230—400 mesh ASTM; Merck) were used, while for TLC and p-TLC, DC-Fertigplatten SIL-G 25 UV254 (Macherey-Nagel) and Silica gel GF_{254} (Merck) were used. In general, extract solutions were washed with brine, dried over magnesium sulfate, then filtered, and the filtrate was evaporated to dryness under reduced pressure, unless otherwise stated.

Extraction of the Wood of *X. nitidum* The finely chipped wood (4.69 kg) of *X. nitidum*, collected in Taiwan in 1981, was extracted with hot methanol for 10 h. The methanol was evaporated under reduced pressure to give the methanol extract (306 g). A part (205 g) of it was partitioned as shown in Chart 1 to give fr. A (5.04 g), fr. B (8.59 g), and fr. C [fr. C_1 (2.54 g), fr. C_2 (2.41 g), fr. C_3 (11.7 g), and fr. C_4 (25.6 g)].

Treatment of Fr. A Fr. A was separated by column chromatography and p-TLC to give nineteen components, which are shown in order of an increasing polarity: (i) methyl 2,3,4-trimethoxycinnamate (0.00022%), pale yellow needles, mp 52 °C (lit.[13] mp 54—55 °C), (ii) 3,4,5-trimethoxycinnamaldehyde (0.00013%), pale yellow needles, mp 112—114 °C (lit.[14] mp 109—111 °C), (iii) 6,7,8-trimethoxycoumarin (0.00076%), colorless needles, mp 105 °C (lit.[8] mp 107—108 °C), (iv) aesculetin dimethyl ether (0.00955%), pale yellow prisms, mp 147 °C (lit.[7] mp 148—149 °C), (v) methyl nitinoate (**2**) (0.00060%), (vi) 3,4,5-trimethoxycinnamyl alcohol (0.00127%), a pale yellow oil, (vii) wutaiensal (0.00094%), a pale yellow oil,[12] (viii) skimmianine (0.00245%), pale yellow prisms, mp 176 °C (lit.[7] mp 173—174.5 °C), (ix) γ-fagarine (0.00197%), pale yellow prisms, mp 148 °C (lit.[7] mp 138—140.5 °C), (x) 4-methoxy-1-methyl-2-quinolone (0.00080%), colorless fine needles, mp 99—103 °C (lit.[8] mp 99—101 °C), (xi) liriodenine (0.00240%), yellow needles, mp 284—285 °C (lit.[8] mp 288—288.5 °C), (xii) chelerythrine chloride (0.00017%), yellow needles, mp 201 °C (lit.[11] mp 200—204 °C), (xiii) isoarnottianamide (0.00076%), pale yellow needles, mp 260 °C (lit.[7] mp 252—256 °C), (xiv) edulutine (0.00054%), pale yellow needles, mp 213 °C (lit.[9] mp 238—239 °C), (xv) dihydrocuspidiol (**3**) (0.00382%), (xvi) ribalinine (0.00028%), pale yellow needles, mp 236 °C (lit.[10] mp 232 °C), (xvii) isoplatydesmine (0.00017%), pale yellow fine needles, mp 189 °C (lit.[10] mp 191 °C), (xviii) oxynitidine (0.00121%), colorless needles, mp 295 °C (lit.[7] mp 288—289.5 °C), (xix) nitidine (**1**) chloride (0.00011%), pale yellow needles, mp 294 °C (lit.[7] mp 275—276 °C).

Methyl Nitinoate (2) Obtained as pale yellow needles, mp 55 °C. IR ν_{max}^{KBr} cm^{-1}: 3524 (OH), 1700 (CO). UV λ_{max}^{MeOH} (log ε): 310 (4.27), 226 (4.05). ^{1}H-NMR δ: 1.00 (3H, d, J=6.9 Hz, $C_{3'}$-Me), 1.65—1.69 (1H, m, $C_{2'}$-H), 1.90—1.97 (2H, m, $C_{2'}$-, $C_{3'}$-H), 3.55 (2H, d, J=5.5 Hz, $C_{4'}$-H_2), 3.79 (3H, s, OMe), 4.04—4.10 (2H, m, $C_{1'}$-H_2), 6.30 (1H, d, J=16 Hz, C_8-H), 6.89 (2H, d, J=8.7 Hz, C_2-, C_6-H), 7.46 (2H, d, J=8.7 Hz, C_3-, C_5-H), 7.64 (1H, d, J=16 Hz, C_7-H). ^{13}C-NMR δ: 16.7 ($C_{3'}$-Me), 32.6 ($C_{2'}H_2$), 33.1 ($C_{3'}$H), 51.5 (OMe), 66.2 ($C_{1'}H_2$), 67.9 ($C_{4'}H_2$), 114.8 (C_2, C_6H), 115.2 (C_8H), 127.1 (C_4), 129.7 (C_3, C_5H), 144.5 (C_7H), 160.6 (C_1), 167.8 (C_9). ORD (c=0.23, MeOH) [α] (nm): 4.34 (500), 13.0 (400). EI-MS m/z: 264 (7.5%, M^+), 178 (100%). This was identical with a sample of methyl nitinoate (**2**) prepared as described below.

Dihydrocuspidiol (3) Obtained as pale yellow needles, mp 52 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3446 (OH). ^{1}H-NMR δ: 0.96 (3H, d, J=6.8 Hz, $C_{3'}$-Me), 1.62−1.65 (1H, m, $C_{2'}$-H), 1.80—1.90 (6H, m, C_8-H_2, $C_{2'}$-, $C_{3'}$-H, OH×2), 2.61 (2H, t, J=7.6 Hz, C_7-H_2), 3.51 (2H, d, J=5.4 Hz, $C_{4'}$-H_2), 3.63 (2H, t, J=6.5 Hz, C_9-H_2), 3.95—4.02 (2H, m, $C_{1'}$-H_2), 6.80 (2H, d, J=8.8 Hz, C_2-, C_6-H), 7.07 (2H, d, J=8.8 Hz, C_3-, C_5-H). HR-FAB-MS m/z: 239.1638 (Calcd for $C_{14}H_{23}O_3$: 239.1647). This was identical with a sample prepared by the reduction of cuspidiol (**8**), in which a solution of **8** (0.031 g, 0.132 mmol) and tris(triphenylphosphine)-chlororhodium (0.010 g, 0.011 mmol) in dry EtOH (1 ml) was hydrogenated at room temperature and at atmospheric pressure for 24 h to give dihydrocuspidiol (**3**) as a colorless oil (0.012 g, 37%).

Synthesis of Methyl Nitinoate (2) (i) Methyl 4-Chloro-2-methylbutanoate: A solution of 2-methyl-γ-lactone (**6**) (1.11 g, 0.011 mol) in dry MeOH (6 ml) was saturated with dry hydrogen chloride under ice-cooling. The mixture was kept to stand at room temperature for 24 h and then extracted with ether. Work-up gave a colorless oil (1.62 g, 97%), which was used without further purification. IR ν_{max}^{neat} cm^{-1}: 1737 (CO). ^{1}H-NMR δ: 1.21 (3H, d, J=7.3 Hz, C_2-Me), 1.81—1.88 (1H, m, C_3-H), 2.15—2.22 (1H, m, C_3-H), 2.71—2.76 (1H, m, C_2-H), 3.57 (2H, dt, J=6.5, 2.0 Hz, C_4-H), 3.70 (3H, s, OMe).

(ii) 4-Chloro-2-methylbutanol: A solution of the ester (1.61 g, 0.011 mol) in dry ether (16 ml) was slowly dropped into a suspension of lithium aluminum hydride (0.41 g, 0.011 mol) in dry ether (16 ml) under ice-cooling, and the mixture was stirred for 2.5 h under the same condition. After decomposition of the excess hydride with 10% H_2SO_4, work-up gave a colorless oil (1.03 g, 69%), which was used without further purification. IR ν_{max}^{neat} cm^{-1}: 3356 (OH). ^{1}H-NMR δ: 0.96 (3H, d, J=6.7 Hz, C_2-Me), 1.61—1.67 (3H, m, C_2-H, OH), 1.86—1.97 (2H, m, C_3-H), 3.53 (2H, d, J=5.8 Hz, C_1-H), 3.56—3.62 (1H, m, C_4-H), 3.62—3.68 (1H, m, C_4-H).

(iii) 4-Chloro-2-methylbutyl Acetate (**7**): A mixture of the alcohol (0.90 g, 7.35 mmol) in acetic anhydride (1.49 ml, 15.7 mmol) and pyridine (0.9 ml) was kept standing at room temperature for 3 h. Work-up gave a colorless oil (0.95 g, 78.6%), which was used without further purification. IR ν_{max}^{neat} cm^{-1}: 1738 (CO). ^{1}H-NMR δ: 0.97 (3H, d, J=6.9 Hz, C_2-Me), 1.61—1.69 (1H, m, C_2-H), 1.87—1.93 (1H, m, C_3-H), 2.05—2.09 (1H, m, C_3-H), 2.07 (3H, s, COMe), 3.54—3.64 (2H, m, C_4-H_2), 3.93 (1H, dd, J=11, 6 Hz, C_1-H), 3.97 (1H, dd, J=11, 6 Hz, C_1-H).

(iv) Methyl Nitinoate (**2**): A mixture of methyl *p*-hydroxycinnamate (**5**) (0.101 g, 0.57 mmol), 4-chloro-2-methylbutyl acetate (**7**) (0.086 g, 0.35 mmol), 3 N KOH (0.68 ml), and potassium iodide (KI) (0.093 g, 0.56 mmol) in dimethyl sulfoxide (2 ml) was stirred at 60 °C for 100 h, during which the chloride (total amount: 0.424 g, 2.57 mmol) and KI (total amount: 0.362 g, 2.18 mmol) were further added at intervals of *ca.* 20 h. After work-up, the crude product was treated with 0.1 N MeONa in MeOH (8.5 ml) for 2 h under ice-cooling and then the mixture was acidified with 1 N HCl. Work-up followed by column chromatography afforded methyl nitinoate (**2**) as colorless needles (from ether–hexane), mp 55 °C. *Anal.* Calcd for $C_{15}H_{20}O_4$; C, 68.15; H, 7.64. Found: C, 68.03; H, 7.68.

Treatment of Fr. B Fr. B was also separated by column chromatography and p-TLC to give two additional components: syringaresinol (0.00189%), colorless prisms, mp 174—181 °C (lit.[8] mp 175.5—180 °C) and nitidanin (**4**) (0.00075%).

Nitidanin (4) Obtained as a colorless powder, mp 232 °C. IR ν_{max}^{KBr} cm^{-1}: 3494 (OH). UV λ_{max}^{MeOH} nm: 224, 272. UV $\nu_{max}^{MeOH+1\%\ NaOH}$ nm: 269. NMR: see Table 1. ORD (c=0.02, MeOH) [α] (nm): 0 (600—400). HR-FAB-MS m/z: 404.1468 (Calcd for $C_{21}H_{24}O_8$: 404.1471).

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16) From the same non-alkaloidal fraction (fr. C) of the bark of this

plant, six known components (chelerythrine,[11] decarine,[6] arnottianamide,[11] piperonylic acid, asarinin,[7] and savinin[17]) have been isolated by us (Ishii H., Johji S., Chen I.-S., Ishikawa T., unpublished results).

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