Constituents of the Root Bark of *Murraya paniculata* Collected in Indonesia

Fujio IMAI, Kikuo ITOH, Noriko KISHIBUCHI, Takeshi KINOSHITA*,[1] and Ushio SANKAWA

Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan. *Received July 6, 1988*

A mixture of fatty acid esters (Va) of murrangatin was obtained from the root bark of *Murraya paniculata* (Rutaceae) collected in Indonesia together with eleven known constituents of chemotaxonomical significance, and their structures were characterized on the basis of spectroscopical and chemical data. Neither prenylindoles nor biogenetically related compounds, found in Formosan *M. paniculata*, were detected.

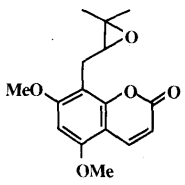
Keywords *Murraya paniculata*; Rutaceae; coumarin; prenylcoumarin; murrangatin fatty acid ester; murrangatin palmitate; LSPD

Introduction

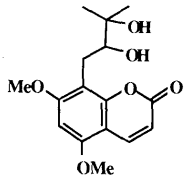
In a previous paper[2] we reported the isolation of three 3-prenylindoles and some coumarins from the root bark of *Murraya paniculata* (L.) JACK collected in Taiwan. In a continuation of this research we examined the same species of Indonesian origin which differed chemically from the Formosan species in the preliminary microchemical analysis. In this paper we describe the isolation of prenylcoumarins, flavones and alkaloids, which were mostly absent in Formosan variety, from the root bark of *M. paniculata* collected in Indonesia.

Results and Discussion

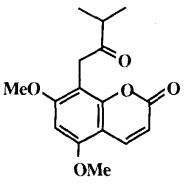
The separation of the chloroform extract of *M. paniculata* root bark collected in Indonesia was carried out according to the scheme shown in Charts 1 and 2 to yield compounds I—XII. Compounds I—IV, VI, VII and IX were readily elucidated as (−)-sibiricin,[3] mexoticin,[4]



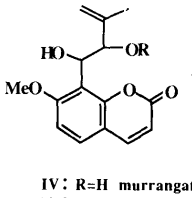
sibiricin (I)



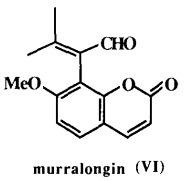
mexoticin (II)



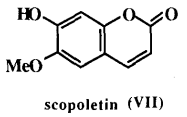
III



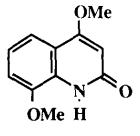
IV: R=H murrangatin
Va: R=CO(CH$_2$)$_{11}$CH$_3$
Vb: R=CO(CH$_2$)$_{14}$CH$_3$



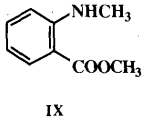
murralongin (VI)



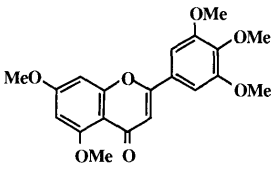
scopoletin (VII)



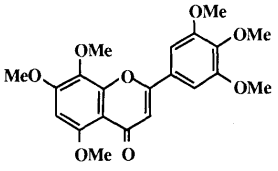
edulitine (VIII)



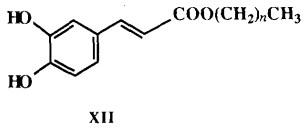
IX



X



XI



XII

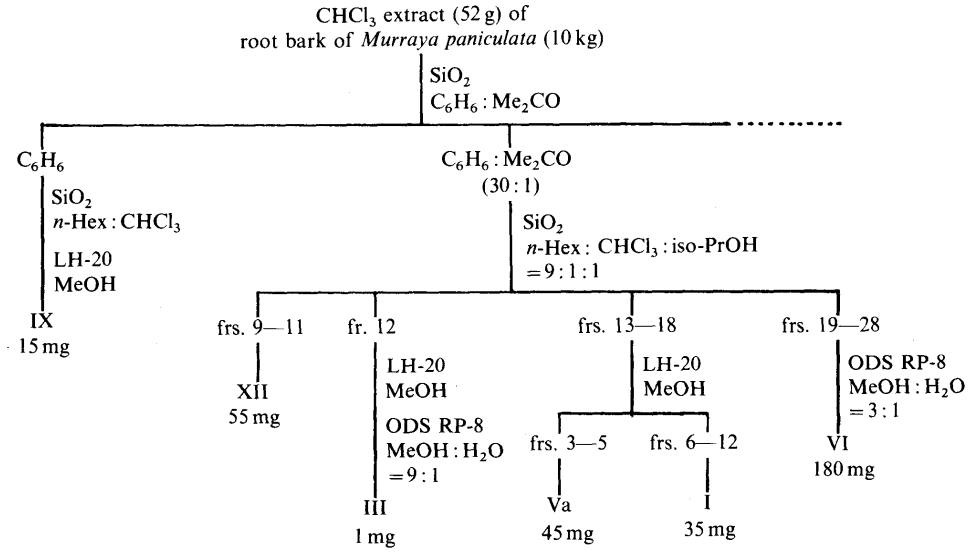


Chart 1. Procedure for Purification of Constituents from *M. paniculata*

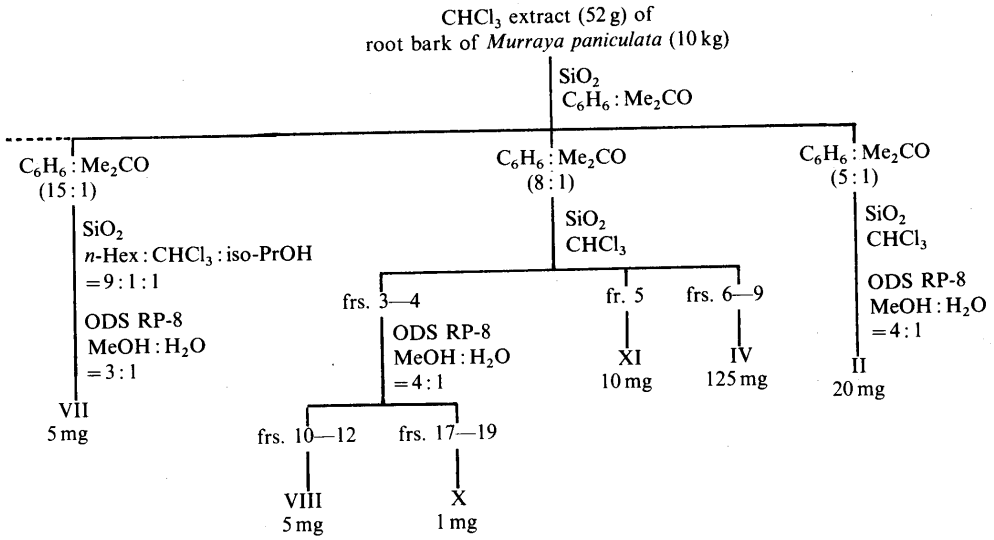


Chart 2. Procedure for Purification of Constituents from *M. paniculata*

5,7-dimethoxy-8-(3-methyl-2-oxobutyl)coumarin,[5] murrangatin,[6] murralongin,[7] scopoletin[8] and methyl *N*-methylanthranylate, respectively, by spectroscopic methods, and these identifications were confirmed by comparison of the spectroscopic data with values reported in the literature.

Compound Va was obtained as a colorless powder. The 7-methoxy-8-substituted coumarin skeleton was indicated by the ultraviolet (UV) [λ_{max} 257, 332 nm] and proton nuclear magnetic resonance (^{1}H-NMR) [δ 7.61 and 6.25 (1H each, d, J=9.5 Hz), 7.39 and 6.87 (1H each, d, J=8.5 Hz), 4.00 (3H, s)] spectra. Signals at δ 1.75 (3H, s), 4.74, 4.77 (1H each, m), 5.53 (1H, d, J=7.9 Hz) and 5.75 (1H, d, J=7.9 Hz) were readily assigned to allyl methyl, *exo*-methylene and glycol protons respectively, suggesting Va to be a murrangatin derivative. The presence of a long-chain fatty acid ester was indicated by signals at δ 0.88 (3H, t, J=7.0 Hz), 1.26 (2*n*H, br s) and 2.39 (2H, t, J=7.5 Hz) in the ^{1}H-NMR spectrum. According to gas chromatography-mass spectrometry analysis of the methanolysate of this compound, palmitate predominated, accounting for 55.8% of total fatty acids. Mild acylation of murrangatin with palmitoyl chloride provided the monopalmitoyl ester (Vb), which was identical with compound Va in terms of the ^{1}H-NMR spectrum, whereas forcing conditions afforded the dipalmitoyl ester, implying that the palmitoyl group is located at the sterically less hindered position (2′-C). The location of the palmitoyl group was unequivocally determined to be at the 2′-position on the basis of a long-range selective decoupling (LSPD) experiment. Mild irradiation of the proton giving the signal at δ 5.75 (2′-H) eliminated the splitting of the non-decoupled carbon signal at δ 172.8 (C=O), whereas irradiation of the proton giving the signal at δ 5.53 (1′-H) produced no significant change in the multiplicity of the same carbon signal. The assignment of 2′-H was confirmed by the ^{1}H–^{1}H correlation spectroscopy (COSY), ^{13}C–^{1}H COSY and long-range ^{13}C–^{1}H COSY spectra as illustrated in Fig. I. Recently, murrangatin acetate was isolated from *Murraya exotica*, and its structure was empirically elucidated based on the downfield shift of a proton attached to a carbon bearing an acetoxy

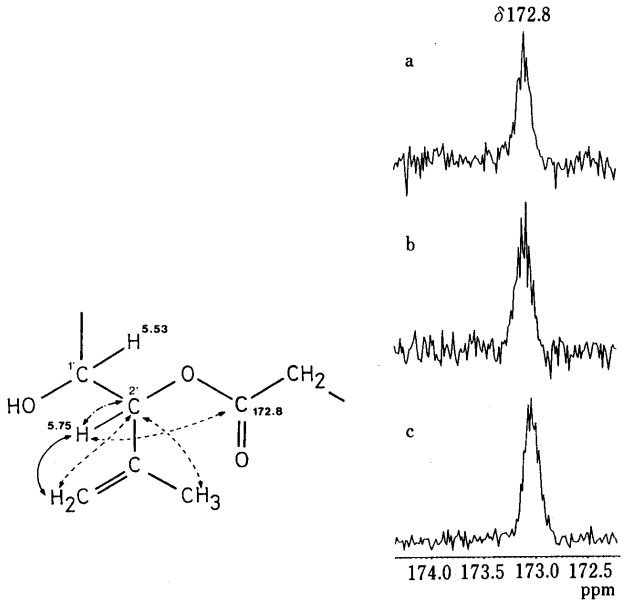


Fig. 1. Nondecoupled and LSPD Spectra of Murrangatin Palmitate (Vb)

The assignment of 2′-H was confirmed by ^{1}H–^{1}H (——), ^{1}H–^{13}C (—·—) and long-range ^{1}H–^{13}C (– – –) shift-correlated 2D-NMR. a, irradiated at 2′-H (5.75); b, irradiated at 1′-H (5.53); c, nondecoupled.

group.[8] Since the chemical shifts of 1′- and 2′-H of murrangatin acetate are in good agreement with those of murrangatin palmitate, the results of LSPD experiment on compound Vb further substantiate the structure of murrangatin acetate, too.

Compound VIII was obtained as colorless prisms, mp 238—239 °C. The molecular formula $C_{11}H_{11}NO_3$ was indicated by the elementary analysis (Calcd: C, 64.38, H. 5.40, N, 6.83. Found: C, 64.58, H, 5.33, N, 6.73) and mass spectrum (MS) [*m/z*: 205 (M^+)]. The 2-quinolone skeleton was suggested by the infrared (IR) [ν_{max} 3140, 1630 cm^{-1}] and UV (λ_{max} 248, 272, 283 and 324 nm) spectra. The ^{1}H-NMR spectrum revealed the presence of a 1,2,3-trisubstituted aromatic system, two aromatic methoxy groups and two singlets, one of which was exchangeable with D_2O. These spectroscopic findings led to the structure VIII for

this compound. A search of the literature revealed that compound VIII is identical to edulitine, which was first isolated from the rutaceous plant *Casimiroa edulis*[9] and for which detailed spectral data are not currently available. The isolation of edulitine from *M. paniculata* is of chemotaxonomical significance since it is the first instance of isolation from the genus *Murraya*.

Compounds X and XI both exhibited a positive Shinoda reaction (pale red), indicating that they are flavone derivatives. Compound X was obtained as colorless needles, mp 197 °C. The ^{1}H-NMR spectrum revealed the presence of five methoxyls, two identical aromatic singlets and a pair of AB doublets, which readily allowed assignment of the structure as 3′,4′,5,5′,7-pentamethoxyflavone.[10] Compound XI, mp 195—196 °C, was also shown to be a polymethoxyflavone with one additional methoxy at either the 6- or the 8-position of X on the basis of the ^{1}H-NMR and MS spectra and was finally identified as 3′,4′,5,5′,7,8-hexamethoxyflavone by comparison of the spectral data with those reported in the literature.[11] Both compounds have also been isolated from *M. paniculata*.[10,11] However, the structures of these compounds were elucidated only on the basis of the spectroscopic data. Therefore chemical syntheses of compounds X and XI were carried out according to the Baker–Venkataraman method, and thus their structures were confirmed.

Compound XII was obtained as a colorless powder. The IR spectrum suggested the presence of hydroxyl, α,β-unsaturated ester, aromatic ring and polymethylene groups. The ^{1}H-NMR spectrum showed the presence of primary methyl at δ0.88 (3H, t, J=5.2 Hz), a polymethylene group at δ1.29 (2nH, br s), –COOCH$_2$– at δ4.16 (2H, t, J=6.5 Hz), ABX-type protons on the aromatic ring and a pair of doublets (J=16 Hz) at δ6.27 and 7.54. On hydrolysis with alkali, compound XII afforded caffeic acid and a mixture of $C_{20}H_{41}OH$ and $C_{22}H_{45}OH$, the ratio of which was shown to be 3:7 by GC. From the above data this compound was formulated as XII. Recently, a long-chain aliphatic alcohol ester of caffeic acid was reported by Komatsu *et al.* from *Sophora tomentosa*.[12]

It is noteworthy that there is significant chemical difference between the Indonesian variety and the Formosan variety of *M. paniculata* though no morphological distinction was visible.[13] Interestingly, 3-prenylindoles or biogenetically related compounds, which are main constituents in the root bark of Formosan *M. paniculata*, could not be detected in Indonesian *M. paniculata*, and instead 8-substituted 5,7-dimethoxycoumarins that were absent in Formosan *M. paniculata* were isolated. These findings imply the presence of at least two distinct chemotypes in *M. paniculata* depending on its geographical location, though further investigation is required to confirm this.

Experimental

All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Spectral data were obtained using the following apparatus: ^{1}H- and ^{13}C-NMR spectra with JEOL FX-100 (^{1}H, 100 MHz; ^{13}C, 25 MHz) and JEOL JMN GX-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometers with tetramethylsilane (TMS) as an internal standard; MS with a JEOL JMS-DX300 mass spectrometer; IR spectra with a JASCO DS-701G spectrometer; UV spectra with a Hitachi spectrophotometer, model 100-60. Optical rotations were measured with a JASCO DIPO-140 digital polarimeter. GC-MS was run on a Shimadzu GCMS-QP1000 using a capillary column packed with OV-1. GC was run on a Shimadzu GC-15A PFsc. Column chromatography was carried out with Wakogel C-200 or Kieselgel 60. Thin-layer chromatography (TLC) was conducted on 0.25 mm precoated silica gel (60F$_{254}$, Merck), and spots were detected under UV light (254 or 360 nm) or by spraying 10% H_2SO_4 followed by heating.

Plant Material *M. paniculata* was collected in 1984 through 1985 in its natural habitat near Skabumi, Java, Indonesia.

Extraction and Isolation The dried root bark (10 kg) of *M. paniculata* was extracted with chloroform at room temperature. The chloroform extract was evaporated to dryness under reduced pressure to give a thick brown gum (52 g). The whole extract was subjected to silica gel column chromatography. Successive elution with benzene, benzene–acetone (30:1), benzene–acetone (15:1), benzene–acetone (8:1) and benzene–acetone (5:1) gave five fractions. Each fraction was further subjected to a combination of silica gel, reverse-phase silica gel and Sephadex LH-20 column chromatographies to furnish twelve constituents in pure forms. The isolation and purification procedure of each constituent are outlined in Charts 1 and 2.

Sibiricin (I) Colorless needles from MeOH, mp 149—150 °C. $[\alpha]_D^{20}$ −70° ($CHCl_3$, c=0.10). UV λ_{max}^{EtOH} nm (log ε): 252 (3.91), 258 (3.95), 327 (4.13). IR ν_{max}^{KBr} cm^{-1}: 1714, 1600, 1245. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.27, 1.45 (3H each, s, 4′- and 5′-Me), 2.85—3.17 (3H, m, 1′- and 2′-H), 3.94 (6H, s, 2×OMe), 6.14 (1H, d, J=9.5 Hz, 3-H), 6.34 (1H, s, 6-H), 8.00 (1H, d, J=9.5 Hz, 4-H). EIMS m/z (rel. int., %): 290 (M$^+$, 11), 247 (20), 232 (17), 220 (27), 219 (M$^+$ −(CH$_3$)$_2$COCH, 100), 217 (17), 189 (18), 161 (38).

Mexoticin (II) Colorless needles from $CHCl_3$–Et_2O, mp 186—188 °C. $[\alpha]_D^{20}$ +50° ($CHCl_3$, c=0.16). UV λ_{max}^{EtOH} nm (log ε): 262 (3.99), 328 (4.13). IR ν_{max}^{KBr} cm^{-1}: 3420, 2960, 1708, 1600, 1432, 1325, 1252, 1230. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.32 (6H, s, 4′- and 5′-Me), 2.29 (2H, br s, 2×OH), 2.90 (1H, dd, J=14.0, 9.0 Hz, 1′-H(H)), 3.00 (1H, dd, J=14.0, 3.5 Hz, 1′-H(H)), 3.60 (1H, dd, J=9.0, 3.5 Hz, 2′-H), 3.94 (6H, s, 2×OMe), 6.14 (1H, d, J=9.5 Hz, 3-H), 6.35 (1H, s, 6-H), 7.99 (1H, d, J=9.5 Hz, 4-H). EIMS m/z (rel. int., %): 308 (M$^+$, 1), 250 (20), 249 (M$^+$ −(CH$_3$)$_2$C(OH), 21), 221 (6), 219 (M$^+$ −(CH$_3$)$_2$C(OH)–CH(OH), 61), 208 (13), 207 (M$^+$ −(CH$_3$)$_2$C(OH)–CH(OH)–CH$_2$, 100), 205 (12), 161 (38).

5,7-Dimethoxy-8-(3-methyl-2-oxo-butyl)coumarin (III) Colorless needles from MeOH, mp 121—122 °C. UV λ_{max}^{EtOH} nm: 237, 250, 257, 322. IR ν_{max}^{KBr} cm^{-1}: 1710, 1600, 1500, 1460, 1450, 1428. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.20 (6H, d, J=7.0 Hz, Me×2), 2.78 (1H, sept, J=7.0 Hz, 3′-H), 3.85 (3H, s, OMe), 3.90 (2H, s, 1′-H$_2$), 3.94 (3H, s, OMe), 6.16 (1H, d, J=9.5 Hz, 3-H), 6.30 (1H, s, 6-H), 7.96 (1H, d, J=9.5 Hz, 4-H). EIMS m/z (rel. int., %): 290 (M$^+$, 11), 220 (28), 219 (M$^+$ −(CH$_3$)$_2$CHCO, 100), 205 (M$^+$ −(CH$_3$)$_2$CHCOCH$_2$, 11), 161 (16).

Murrangatin (IV) Colorless granules from benzene–Et_2O, mp 132 °C, $[\alpha]_D^{20}$ +7.4° ($CHCl_3$, c=0.244). UV λ_{max}^{EtOH} nm (log ε): 250 (3.57), 258 (3.60), 322 (4.17). IR ν_{max}^{KBr} cm^{-1}: 3480, 2940, 1710, 1600, 1560, 1492, 1400, 1245. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.77 (3H, s, 5′-Me), 3.45 (2H, br s, 2×OH), 3.97 (3H, s, OMe), 4.57 (1H, d, J=8.5 Hz, 2′-H), 4.60 (2H, m, 4′-H), 5.30 (1H, d, J=8.5 Hz, 1′-H), 6.24 (1H, d, J=9.5 Hz, 3-H), 6.88 (1H, d, J=8.5 Hz, 6-H), 7.40 (1H, d, J=8.5 Hz, 5-H), 7.63 (1H, d, J=9.5 Hz, 4-H). ^{13}C-NMR (25 MHz, $CDCl_3$) δ: 17.4 (5′-C), 56.2 (OMe), 69.5 (1′-C), 78.2 (2′-C), 107.6 (6-C), 112.9 (8- or 4a-C), 113.2 (3-C), 113.4 (4′-C), 115.9 (4a- or 8-C), 128.3 (5-C), 143.4 (4-C), 143.7 (3′-C), 152.7 (8a-C), 159.7 (2- or 7-C), 159.9 (7- or 2-C). EIMS m/z (rel. int., %): 259 (M$^+$ −OH, 1), 220 (28), 206 (21). 205 (M$^+$ −CH$_2$=C(CH$_3$)–CHOH, 100), 175 (15).

Murrangatin Ester (Va) Colorless powder from n-hexane. UV λ_{max}^{EtOH} nm: 246, 257, 332. IR ν_{max}^{KBr} cm^{-1}: 3540, 1725, 1605, 1245. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.88 (3H, t, J=7.0 Hz, –(CH$_2$)$_n$C<u>H</u>$_3$), 1.26 (br, –(C<u>H</u>$_2$)$_n$–), 1.75 (3H, s, 5′-Me), 2.39 (2H, t, J=7.5 Hz, –OCOCH$_2$), 4.00 (3H, s, OMe), 4.74 (1H, m, 4′-H), 4.77 (1H, m, 4′-H), 5.53 (1H, d, J=7.9 Hz, 1′-H), 5.75 (1H, d, J=7.9 Hz, 2′-H), 6.25 (1H, d, J=9.5 Hz, 3-H), 6.87 (1H, d, J=8.5 Hz, 6-H), 7.39 (1H, d, J=8.5 Hz, 5-H), 7.61 (1H, d, J=9.5 Hz, 4-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.10 (–(CH$_2$)$_n$<u>C</u>H$_3$), 18.5 (5′-C), 20.32 (–(C<u>H</u>$_2$)$_n$–), 34.5 (OCO<u>C</u>H$_2$), 56.3 (OMe), 68.2 (1′-C), 79.2 (2′-C), 107.8 (6-C), 113.0 (8- or 4a-C), 113.4 (3-C), 114.7 (4′-C), 115.8 (4a- or 8-C), 128.8 (5-C), 140.9 (3′-C), 143.7 (4-C), 152.7 (8a-C), 160.0 (2- or 7-C), 160.2 (7- or 2-C), 173.7 (O<u>C</u>OCH$_2$).

Murralongin (VI) Cololess needles from Et_2O, mp 137—138 °C. UV λ_{max}^{EtOH} nm (log ε): 235 (4.20), 324 (4.19). IR ν_{max}^{KBr} cm^{-1}: 1725 (CHO), 1655, 1600, 1490. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.79 (3H, s, Me), 2.43 (3H, s, Me), 3.84 (3H, s, OMe), 6.23 (1H, d, J=9.5 Hz, 3-H), 6.90 (1H, d, J=8.5 Hz, 6-H), 7.45 (1H, d, J=8.5 Hz, 5-H), 7.65 (1H, d, J=9.5 Hz, 4-H), 10.22 (1H, s, CHO). ^{13}C-NMR (25 MHz, $CDCl_3$) δ: 19.8 (Me), 24.8

(Me), 56.2 (OMe), 107.6 (6-C), 112.8 (8-C, 4a-C and 3-C), 115.9 (4a- or 8-C), 128.5 (5-C), 143.6 (4-C), 152.8 (8a-C), 159.5 (2- or 7-C), 159.8 (7- or 2-C), 160.9 (CHO). EIMS *m/z* (rel. int., %): 258 (M^+, 100), 229 (21), 215 (94), 214 (24), 201 (27), 199 (40), 187 (57).

Scopoletin (VII) Colorless needles from $CHCl_3$, mp 205—207 °C. UV λ_{max}^{EtOH} nm (log ε): 253 (3.70), 297 (3.71), 346 (4.09). IR ν_{max}^{KBr} cm^{-1}: 3330, 1700, 1605, 1560, 1500, 1432. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 3.90 (3H, s, OMe), 6.18 (1H, d, J=9.5 Hz, 3-H), 6.80 (1H, s, 5-H), 7.20 (1H, s, 8-H), 7.85 (1H, d, J=9.5 Hz, 4-H), 8.83 (1H, br, –OH). EIMS *m/z* (rel. int., %): 192 (M^+, 100), 177 (M^+–Me, 66), 164 (M^+–CO, 66), 149 (M^+–Me–CO, 66), 121 (33).

Edulitine (VIII) Colorless needles from $CHCl_3$–Et_2O, mp 238—239 °C. UV λ_{max}^{EtOH} nm (log ε): 248 (4.46), 272 (3.89), 283 (3.87), 324 (3.50). IR ν_{max}^{KBr} cm^{-1}: 3140, 1630, 1568, 1478, 1450, 1388, 1265, 1228, 1078. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 3.97 (6H, s, 2×OMe), 6.00 (1H, s, 3-H), 6.97 (1H, dd, J=8.0, 1.7 Hz, 7-H), 7.14 (1H, t, J=8.0 Hz, 6-H), 7.48 (1H, dd, J=8.0, 1.7 Hz, 5-H), 9.20 (1H, br, exchangeable with D_2O, –NH). EIMS *m/z* (rel. int., %): 205 (M^+, 100), 204 (74), 190 (20), 176 (39), 175 (39), 162 (14). *Anal.* Calcd for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40; N, 6.83. Found: C, 64.58; H, 5.33; N, 6.73.

Methyl *N*-Methyl Anthranylate (XI) Pale yellow oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 2940, 1676, 1605, 1580, 1515, 1437, 1260, 1245. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 2.91 (3H, s, N-Me), 3.85 (3H, s, $COOCH_3$), 6.58 (1H, ddd, J=8.4, 7.0, 1.0 Hz, 5-H), 6.66 (1H, dd, J=8.4, 1.0 Hz, 3-H), 7.38 (1H, ddd, J=8.4, 7.0, 1.7 Hz, 4-H), 7.89 (1H, dd, J=8.0, 1.7 Hz, 6-H). EIMS *m/z*: 165 (M^+).

3′,4′,5,5′,7-Pentamethoxyflavone (X) Colorless needles from benzene–Et_2O, mp 197 °C. UV λ_{max}^{EtOH} nm (log ε): 267 (4.20), 324 (4.29). IR ν_{max}^{KBr} cm^{-1}: 2920, 1630, 1600, 1450, 1415, 1345, 1240. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 3.9—4.1 (15H, 5×OMe), 6.40 (1H, br s, 3-H), 6.60 (1H, br s, 6-H), 6.70 (1H, s, 8-H), 7.10 (2H, s, 2′- and 6′-H). EIMS *m/z* (rel. int., %): 372 (M^+, 100). 355 (7), 343 (20), 342 (7), 341 (12), 327 (13), 326 (20), 205 (11).

3′,4′,5,5′,7,8-Hexamethoxyflavone (XI) Colorless needles from MeOH. mp 195—196 °C. UV λ_{max}^{EtOH} nm (log ε): 273 (4.19), 310 sh (4.10), 330 (4.12). IR ν_{max}^{KBr} cm^{-1}: 2920, 1635, 1600, 1500, 1420, 1345, 1240, 1210. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 3.9—4.0 (18H, 6×OMe), 6.45 (1H, s, 3-H), 6.65 (1H, s, 6-H), 7.19 (2H, s, 2′- and 6′-H). EIMS *m/z* (rel. int., %): 402 (M^+, 47), 387 (M^+ −15, 57), 358 (36), 291 (45), 205 (11).

Caffeic Acid Ester (XII) Colorless powder from MeOH. UV λ_{max}^{EtOH} nm: 237 sh, 246, 305 sh, 332. IR ν_{max}^{KBr} cm^{-1}: 3470, 3320, 1687, 1636, 1600, 1273. ^{1}H-NMR (100 MHz, d_6-acetone) δ: 0.88 (3H, t, J=5.2 Hz, Me), 1.29 (br s, $-(CH_2)_n-$), 4.16 (2H, t, J=6.5 Hz, $-COOCH_2-$), 6.27 (1H, d, J=16.0 Hz, –CH=), 6.85 (1H, d, J=8.1 Hz, 5-H), 7.03 (1H, dd, J=8.1, 2.0 Hz, 6-H), 7.16 (1H, d, J=2.0 Hz, 2-H), 7.54 (1H, d, J=16.0 Hz, –CH=), 8.25 (1H, s, –OH), 8.49 (1H, s, –OH).

Hydrolysis of XII A mixture of XII (20 mg) and 5% methanolic KOH (10 ml) was heated at 60 °C for 5 min, and extracted with *n*-hexane (10 ml). The *n*-hexane layer was subjected to GC-MS analysis, which identified the aliphatic alcohol part as $C_{20}H_{41}OH$ and $C_{22}H_{45}OH$. The GC-MS was run under the following conditions: column, i.d. 2.6 mm×2 m Shimalite w(AW-DCMS) coated with Silicone OV-1 (2%); column temperature, 240 °C; carrier gas, helium (flow rate; 20 ml/min). In order to obtain the ratio of aliphatic alcohols, the mixture was subjected to GC [column, fused silica capillary OV-1 (i.d. 0.25 mm×25 m); column temperature, 240 °C, carrier gas, N_2 (flow rate, 1 ml/min)], and it was calculated as 30 : 70 for $C_{20}H_{41}OH$ and $C_{22}H_{45}OH$. Retention times were 11.3 and 18.3 min, respectively. The H_2O layer was neutralized with diluted HCl and extracted with AcOEt. After evaporation of the solvent, the residue was recrystallized from aqueous methanol to give a yellow powder, which was identified by direct comparison with authentic caffeic acid.

Alkaline Hydrolysis of Va A mixture of Va (25 mg) and 5% methanolic KOH (15 ml) was stirred overnight at room temperature. The reaction mixture was diluted with 20 ml of water, and then extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was combined, washed successively with 5% Na_2CO_3 and water, dried over Na_2SO_4, and evaporated to dryness. The residue was subjected to preparative TLC to afford murrangatin (8 mg) in a pure form. mp 131—132 °C (benzene–Et_2O). $[\alpha]_D^{21}$ +8.5° ($CHCl_3$, c=0.10). It was identical with the product obtained above in terms of IR and NMR spectra and mixed melting point determination.

GC-MS Analysis of Fatty Acid Methyl Esters Derived from Va A mixture of Va (2 mg) and 10% methanolic hydrogen chloride (2 ml) was warmed at 60 °C for 5 h, and then filtered. The filtrate was evaporated to dryness, redissolved in *n*-hexane, and subjected to GC-MS analysis. The GC-MS analysis was carried out under the following conditions: column, Silicone OV-1 (i.d. 3 mm×2 m); column temperature, 160—200 °C (increased at the rate of 4 °C/min); carrier gas, helium (flow rate, 20 ml/min), and the components were identified as $C_{15}H_{31}COOMe$ (a), anteiso-$C_{16}H_{33}COOMe$+$C_{16}H_{31}COOMe$ (b), $C_{16}H_{33}COOMe$ (c), anteiso-$C_{17}H_{35}COOMe$ (d), $C_{17}H_{35}COOMe$ (e), anteiso-$C_{18}H_{37}COOMe$+$C_{18}H_{35}COOMe$+$C_{18}H_{33}COOMe$ (f) and $C_{18}H_{37}COOMe$ (g), the ratio of which was calculated by GC as 1.1 : 1.5 : 55.8 : 7.2 : 2.1 : 24.0 : 7.1. GC was run under the following conditions: column, capillary G-SCOT OV-1 (i.d. 0.28 mm×20 m); column temperature, 190 °C; carrier gas, N_2 (flow rate, 1 ml/min), and retention times of fatty acid esters were as follows; a, 3.6 min; b, 4.7—4.8 min; c, 5.4 min; d, 7.2 min; e, 8.1 min; f, 9.6-10.6 min; g, 12.0 min.

Preparation of Murrangatin Palmitate (Vb) A mixture of murrangatin (20 mg), palmitoyl chloride (50 mg) and dry pyridine (1 ml) was stirred for 1 h at room temperature, and then the reaction was stopped by the addition of water. The mixture was evaporated to dryness and subjected to preparative TLC (benzene : acetone = 9 : 1) to afford the monopalmitoyl ester as a major product. The monopalmitoyl ester was recrystallized from *n*-hexane to give colorless granules, mp 77 °C. UV λ_{max}^{EtOH} nm (log ε): 248 (3.56), 258 (3.56), 323 (4.16). IR ν_{max}^{KBr} cm^{-1}: 3540, 1725, 1605, 1245. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.86 (3H, t, J=7.0 Hz, $-(CH_2)_{14}\underline{CH_3}$), 1.26 (br, $-(CH_2)_{14}-$), 1.74 (3H, s, 5′-Me), 2.39 (2H, t, J=7.5 Hz, $-OCO\underline{CH_2}$), 4.00 (3H, s, OMe), 4.73 (1H, m, 4′-H), 4.77 (1H, m, 4′-H), 5.53 (1H, d, J=7.9 Hz, 1′-H), 5.76 (1H, d, J=7.9 Hz, 2′-H), 6.24 (1H, d, J=9.5 Hz, 3-H), 6.89 (1H, d, J=8.5 Hz, 6-H), 7.41 (1H, d, J=8.5 Hz, 5-H), 7.63 (1H, d, J=9.5 Hz, 4-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.0 ($-(CH_2)_{14}\underline{C}H_3$), 18.5 (5′-C), 20—32 ($-\underline{C}H_2)_{14}-$), 34.4 ($OCO\underline{C}H_2$), 56.0 (OMe), 68.0 (1′-C), 78.9 (2′-C), 107.3 (6-C), 112.6 (8- or 4a-C), 112.9 (3-C), 114.1 (4′-C), 115.5 (4a- or 8-C), 128.1 (5-C), 140.4 (3′-C), 143.0 (4-C), 152.1 (8a-C), 159.1 (2- or 7-C), 159.6 (7- or 2-C), 172.8 ($O\underline{C}OCH_2$). $[\alpha]_D^{27}$ −12.0° ($CHCl_3$, c=0.13). *Anal.* Calcd for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found: C, 72.67; H, 8.94. When the above reaction was performed at 60 °C, the dipalmitoyl ester was obtained in amorphous form. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.87 (3H, t, J=7.0 Hz, 2×$-(CH_2)_{14}\underline{CH_3}$), 1.25 (br, 2×$-(CH_2)_{14}-$), 1.75 (3H, s, 5′-Me), 2.39 (4H, m, 2×$-OCOCH_2$), 3.95 (3H, s, OMe), 4.72 (1H, m, 4′-H), 4.90 (1H, m, 4′-H), 6.09 (1H, d, J=9.2 Hz, 1′-H), 6.25 (1H, d, J=9.5 Hz, 3-H), 6.65 (1H, d, J=9.2 Hz, 2′-H), 6.82 (1H, d, J=8.4 Hz, 6-H), 7.38 (1H, d, J=8.4 Hz, 5-H), 7.57 (1H, d, J=9.5 Hz, 4-H).

Synthesis of 3′,4′,5,5′,7-Pentamethoxyflavone (X) A mixture of 2,4-dimethoxy-6-hydroxyacetophenone (0.9 g) and 3,4,5-trimethoxybenzoyl chloride (1.2 g) in pyridine (10 ml) was stirred overnight, and the solvent was removed under reduced pressure. The residue was recrystallized from aqueous methanol to give the 3,4,5-trimethoxybenzoyl ester of the acetophenone (1.27 g). The benzoyl ester (1.1 g) and KOH (12 g) in dry pyridine (12 ml) were stirred at room temperature under argon for 48 h, and after acidification with 1 M hydrochloric acid the reaction mixture was extracted with ethyl acetate. The organic layer was washed with 5% Na_2CO_3 to remove by-products resulting from hydrolysis, and then washed with water, dried, and evaporated. The residue was recrystallized from aqueous methanol to give 3′,4′,5,5′,7-pentamethoxyflavone, which was identical with the material isolated from the plant. mp 197—198 °C.

Synthesis of 3′,4′,5,5′,7,8-Hexamethoxyflavone (XI) 2-Hydroxy-3,4,6-trimethoxyacetophenone was prepared quantitatively by methylation of 2,4-dihydroxy-3,6-dimethoxyacetophenone[14] with diazomethane. A mixture of 2-hydroxy-3,4,6-trimethoxyacetophenone (0.5 g) and 3,4,5-trimethoxybenzoyl chloride (0.6 g) was heated at 60 °C overnight. The mixture was poured into ice-water mixture, and extracted with ethyl acetate. The organic layer was washed with 0.5 N hydrochloric acid, washed with water, dried and evaporated to dryness. The residue was recrystallized from aqueous methanol to give the 3,4,5-trimethoxybenzoyl ester of 2-hydroxy-3,4,6-trimethoxyacetophenone (0.5 g). A mixture of the ester (0.5 g) and KOH (5 g) in dry pyridine (5 ml) was reacted as described above to give 3′,4′,5,5′,7,8-hexamethoxyflavone (0.3 g), which was identical with the natural flavone. mp 195—196 °C.

Acknowledgement We are indebted to Mr. S. Henmi, PT Eisai Indonesia, for collection of plant materials, and Dr. T. Kasama, School of Medicine, University of Tokyo, for GC and GC-MS analyses.

References and Notes

1) Present address: *Faculty of Pharmaceutical Sciences, Teikyo University, 1091–1 Sagamiko-machi, Tsukui-gun, Kanagawa 199–01, Japan.*
2) *a*) T. Kinoshita, S. Tatara and U. Sankawa, *Chem. Pharm. Bull.*, **33**, 1770 (1985); *b*) T. Kinoshita, S. Tatara, F.-C. Ho and U. Sankawa,

Phytochemistry, "in press."
3) D. B. M. Wickramaratne, V. Kumar and S. Balasubramaniam, *Phytochemistry*, **23**, 2964 (1984).
4) D. P. Chakraborty and B. K. Chowdhury, *Tetrahedron Lett.*, **1967**, 3471.
5) J.-S. Yang and Y.-L. Su, *Yaoxue Xuebao*, **18**, 760 (1983).
6) S. K. Talapatra, L. N. Dutta and B. Talapatra, *Tetrahedron*, **29**, 2811 (1973).
7) F. Imai, T. Kinoshita, A. Itai and U. Sankawa, *Chem. Pharm. Bull.*, **34**, 3978 (1986).
8) C. Ito and H. Furukawa, *Chem. Pharm. Bull.*, **35**, 4277 (1987).
9) *a)* J. Iriarte, F. A. Kincl, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.*, **1956**, 4170; *b)* T. P. Toube, J. W. Murphy and A. D. Cross, *Tetrahedron*, **23**, 2061 (1967).
10) J.-S. Yang and M.-H. Du, *Huaxue Xuebao*, **42**, 1308 (1984).
11) J.-S. Yang and M.-H. Du, *Zhiwu Xuebao*, **26**, 184 (1984).
12) M. Komatsu, I. Yokoe and Y. Shirataki, *Chem. Pharm. Bull.*, **26**, 3863 (1978).
13) Voucher specimens of both varieties were deposited at the Herbarium, University Museum, University of Tokyo.
14) N. Morita, *Chem. Pharm. Bull.*, **8**, 66 (1960).