Inhibitors of Prostaglandin Biosynthesis from *Dalbergia odorifera*

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The root heartwood of *Dalbergia odorifera* T. CHEN (Leguminosae) is a Chinese medicinal drug (Japanese name koshinko) used for a stagnant blood syndrome (stagnation of disordered blood; Japanese, oketsu). In addition to 10 known compounds, five new phenolic compounds, isomucronustyrene and hydroxyobtustyrene (cinnamylphenols), (+)-isoduartin (isoflavan), odoriflavene (isoflav-3-ene) and (−)-odoricarpan (pterocarpan) were isolated and their structures were elucidated on the basis of chemical and spectroscopic methods. Of the fifteen compounds isolated, cinnamylphenols, isoflavans, isoflavene and benzoic acid derivative significantly inhibited prostaglandin biosynthesis as well as platelet aggregation induced by arachidonic acid.

Keywords *Dalbergia odorifera*; stagnant blood; cinnamylphenol; isoflavene; isoflavan; pterocarpan; prostaglandin biosynthesis inhibitor; platelet aggregation inhibitor

In our studies in search of biologically active compounds contained in medicinal plants used in traditional medicines, *in vitro* bioassay systems have been extensively used to monitor the biological activities of extracts, fractions and isolated compounds. The *in vitro* bioassay tests are advantageous in biological activity oriented phytochemical studies where a large number of fractions should be tested by bioassay along with phytochemical separation. A bioassay system that tests inhibitory effects on a prostaglandin (PG) biosynthesizing enzyme (PG synthetase) has been extensively used in our studies on medicinal plants,[3] since the inhibition of PG biosynthesis is directly associated with anti-inflammatory[4] and anti-platelet aggregation activities.[5] Since we introduced the strategy using *in vitro* bioassay, a considerable success was obtained in studies using PG synthetase.[3] A variety of phenolic compounds was isolated as potent inhibitors of PG synthetase from *Zingiber officinale*,[3a] *Alpinia officinarum*,[3b] *Arnebia euchroma*,[3c-e] *Ipomoea aquatica*,[3f] *Allium chinense*,[3g] and *Mucuna birdwoodiana*.[3h] The isolated active compounds characteristically have structures to contain phenolic and lipophylic groups in their molecules. The substrate of PG synthetase is unsaturated fatty acids such as arachidonic acid (AA), therefore the enzyme should have affinity to lipophylic groups. The phenol group acts to inhibit the formation of radicals or quenches generated radicals in the endoperoxide synthetase reaction, and the lipophylic group assists in the binding of the inhibitors to the enzyme.

Medicinal drugs used in Japanese Kampo medicine are classified in various categories according to their therapeutic use. Medicinal drugs applied to a syndrome of stagnant blood (stagnation of disordered blood; Japanese, oketsu) have been studied for their inhibitory effect on platelet aggregation.[3g,h,5] It has been recognized that the balance between thromboxane A_2 (TXA_2) and PGI_2 has a very important role in the regulation of blood flow and it is reasonable to investigate the inhibitory effects of this type of drug (Japanese, kuoketsuyaku) to PG synthetase. Peony root bark[6] is representative of the drugs, and its efficacy was reported to be attributable to the inhibition of cyclooxygenase of platelet by a preclinical test.[5a] Oral administration of hot aqueous extract of peony root bark to humans resulted in lowering platelet aggregation in

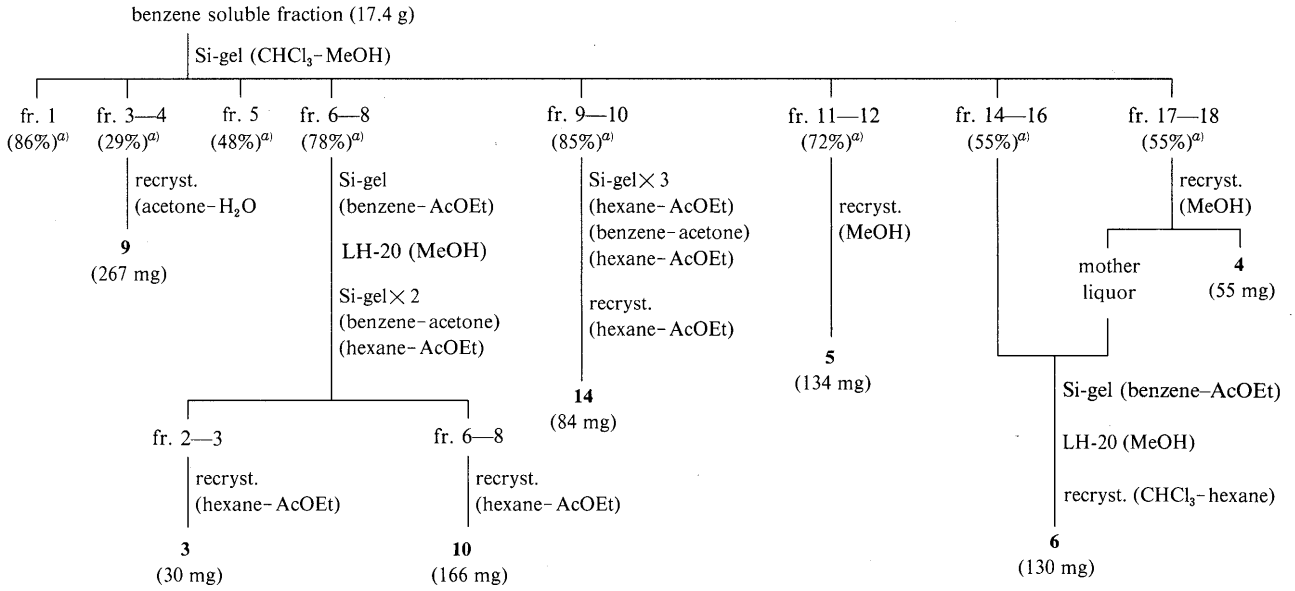

Chart 1. Chromatographic Separation of Benzene Soluble Fraction

a) Figures in parentheses indicate inhibition % for PG synthetase at a concentration of 20 μg/ml.

parallel with a decrease of the activity of TXA_2 synthesis in platelets. An inhibitory effect of the extract on arachidonate cyclooxygenase was seen in a decrease of the level of 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), while the formation of 15-hydroxyeicosatetraenoic acid (15-HETE), a product of lipoxygenase, did not affect paeonol, which is the main constituent of peony root bark and is contained in the aqueous extract, but inhibited PG biosynthesis at a level comparable to aspirin in an *in vitro* assay system used in our studies.[7] The results indicate that paeonol inhibited platelet cyclooxygenase to decrease platelet aggregation induced by arachidonic acid.

Dalbergia odorifera T. CHEN (Leguminosae) is a plant which has been used to treat the stagnation of disordered blood in traditional Chinese medicine.[6] The hot aqueous extract of this medicinal plant was found to inhibit PG biosynthesis by 98% at a concentration of 750 μg/ml. Following this observation we investigated its constituents to clarify the compounds responsible for this activity, and preliminary results have been reported in a previous communication.[5c] This paper deals with details of the study on the biologically active compounds of *D. odorifera*.

The heartwood of *D. odorifera*, purchased in the Taipei market, was extracted with methanol, then the methanol extract was fractionated into hexane-, chloroform- and water-soluble fractions. A benzene soluble fraction obtained from the chloroform soluble fraction had the most potent inhibitory effect on PG synthetase (96% at 150 μg/ml and 60% at 20 μg/ml). A part of the benzene soluble fraction (17.4 g) was repeatedly chromatographed to give seven compounds (Chart 1). During the course of this separation work, the presence of a considerable number of other

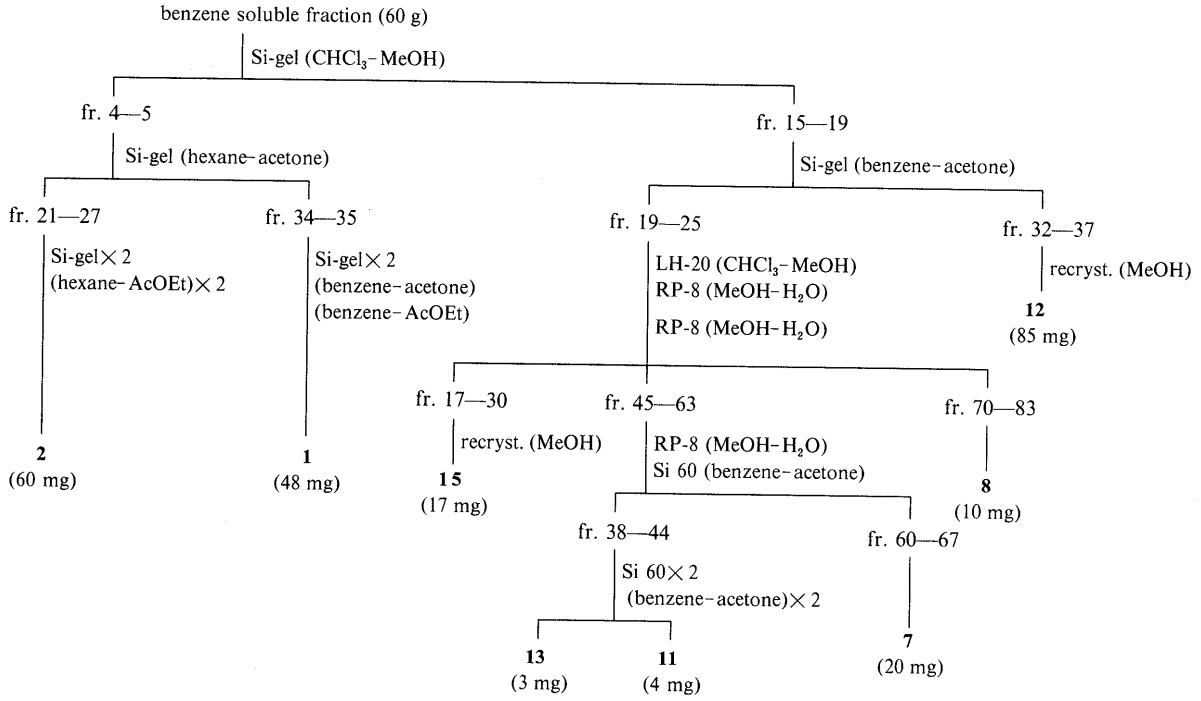

Chart 2. Chromatographic Separation of Benzene Soluble Fraction on Large Scale

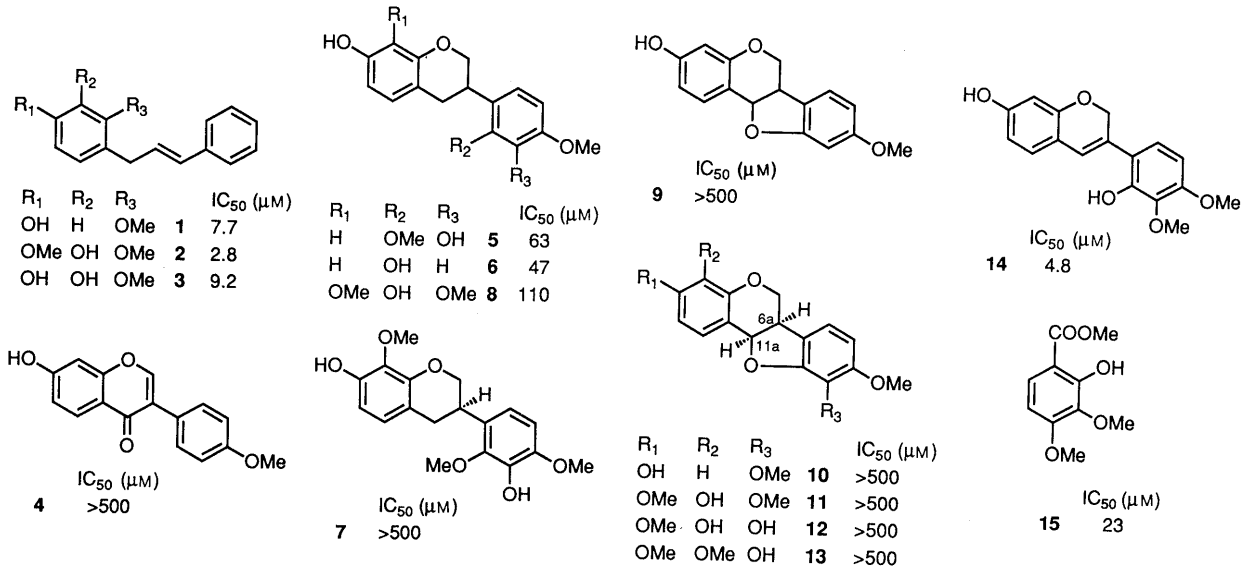

Fig. 1. Structures and Inhibitory Effects of Isolated Compounds

compounds was observed, and we thus further investigated the constituents starting from a larger amount of material. A large-scale chromatographic separation of the benzene soluble fraction (60 g) gave eight further compounds (Chart 2). Of the 15 total compounds isolated, obtustyrene (cinnamylphenol) (**1**),[8] formononetin (isoflavone) (**4**), (±)-mucronulatol (**5**)[9] and (±)-vestitol (isofavan) (**6**),[10] (±)-medicarpin (**9**), (−)-methylnissolin (**10**),[11] (−)-melilotocarpan C (**11**)[12] and (−)-melilotocarpan D (**12**)[12] (pterocarpans) were known compounds, and they were identified by the comparison of their spectral data with those of authentic samples[13] or those reported. The structure of obtustyrene (**1**) was unambiguously established by synthesis from a corresponding chalcone (**16**) (Chart 3).[8b]

Isomucronustyrene (2) The proton nuclear magnetic resonance (^{1}H-NMR) spectrum indicated that it was a new cinnamylphenol substituted with one hydroxyl and two methoxyl groups. One of the methoxyl groups showed a significant benzene-d_6 shift,[14] thus the methoxyl group should be present at the ortho to an aromatic hydrogen. The structure of isomucronustyrene (**2**) was finally confirmed to be *E*-1-(3-hydroxy-2,4-dimethoxybenzyl)-2-phenylethylene (**2**) by its synthesis from 2,6-dimethoxyphenol and cinnamyl alcohol (Chart 3).

Hydroxyobtustyrene (3) The ^{1}H-NMR spectrum indicated that it is a new cinnamylphenol substituted with two hydroxyl and one methoxyl groups. Condensation of cinnamyl alcohol with 1,2-dihydroxy-3-methoxyphenol under an acidic condition gave three reaction products (**3**, **17**, **18**) (Chart 3). Hydroxyobtustyrene (**3**) was identical to *E*-1-(3,4-dihydroxy-2-methoxybenzyl)-2-phenylethylene (**3**) which showed no nuclear Overhauser effect (NOE) upon irradiation of the methoxyl signal.

(+)-Duartin (7) A molecular formula, $C_{18}H_{20}O_6$, was given by a high resolution mass spectrum (HR MS). Its ^{1}H-NMR spectrum was identical to that reported for (−)-duartin which was isolated from *Machaerium opacum*,[15] however, the optical rotatory dispersion (ORD) curve of (−)-duartin was completely opposite of that isolated from *D. odorifera*. Therefore it was identified as (+)-duartin (**7**) of the *R* configuration at C-3.

(±)-Isoduartin (8) It is an optically inactive compound. ^{1}H-NMR and MS are consistent with an isoflavan with an isomeric structure of (+)-duartin (**7**), since the ^{1}H-NMR signals of 5′-H and 6′-H of (±)-isoduartin in chloroform-d_1 showed significant shifts from those of duartin (**7**), and irradiation of one of the methoxyl signals (δ 3.28 in benzene-d_6) caused enhancement of the C-5′ signals (δ 6.10 in benzene-d_6) by 30%, indicating they differ from each other in the substitution of methoxyl and hydroxyl groups at C-2′ and C-3′. Therefore, the structure of (±)-isoduartin (**8**) was established as 7,2′-dihydroxy-8,3′,4′-trimethoxyisoflavan.

(−)-Odoricarpan (13) The molecular formula indicates that it is a pterocarpan and an isomer of melilotocarpan C (**11**).[12] They have the same substitution pattern with one hydroxyl and three methoxyl groups and differ from each other in the positions of methoxyl and hydroxyl groups. Two methoxyl groups of odoricarpan showed benzene-d_6 shifts in ^{1}H-NMR, indicating that two methoxyl groups have hydrogens at the adjacent positions (*ortho*); therefore, 10-hydroxy-3,4,9-trimethoxypterocarpan is the only structure compatible with the spectral data of odoricarpan (**13**). The absolute configurations of C-6a and C-11a were assigned as *R* and *R* by the comparison of ORD data with those of (−)-melilotocarpan C and D (**11** and **12**).[12]

Odoriflavene (14) The ^{1}H-NMR signals are compatible with an isoflav-3-ene with a hydroxyl group at C-7, and one hydroxyl and two methoxyl groups at C-2′, 3′ and 4′. The structure was unambiguously established by direct correlation with a pterocarpan, (−)-methylnissolin (**10**). Catalytic reduction of odoriflavene (**14**) and (−)-methylnissolin (**10**) gave reaction products, (±) and (−)-isomucronulatol (**19**), which gave identical ^{1}H-NMR spectra.

Methyl 2-Hydroxy-3,4-dimethoxybenzoate (15) All spectral data are compatible with a benzoate derivative with one hydroxyl and two methoxyl substitutions. A fragment ion resulting from the loss of CH_4O indicates the presence of hydroxyl group at the adjacent position to carbomethoxyl group.

IC_{50} values of isolated compounds are shown in Fig. 1. The results support our previous observation of phenolic inhibitors of PG synthetase, with those having lipophylic group showing significantly higher inhibitory effects. The results of inhibition experiments of *in vitro* platelet aggregation were summarized in Table I. The most active compounds obtained as the inhibitors of PG synthetase from *D. odorifera* were isomucronustyrene (**2**), hydroxyobtustyrene (**3**) and methyl 2-hydroxy-3,4-dimethoxybenzoate (**15**), which also inhibited platelet aggregation

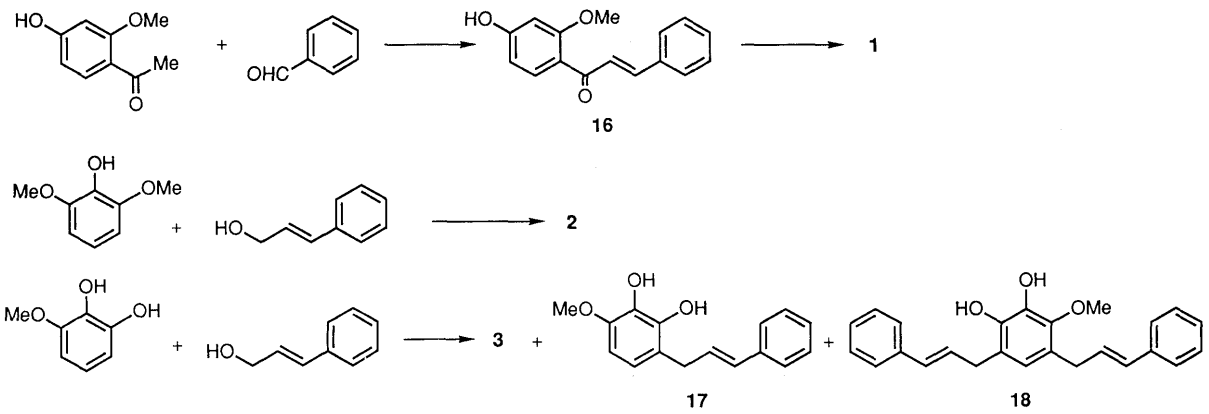

Chart 3. Synthesis of Cinnamylphenols

TABLE I. Inhibitory Effects of Phenolic Compounds Isolated from *Dalbergia odorifera* on Platelet Aggregation Induced by Adenosine Diphosphate (ADP), Arachidonic Acid (AA) and Collagen

	Inducer			
	ADP (10 μM)	AA (128 μM)	Collagen (20 μM)	IC_{50} Values for PG-ase[a]
	Positive control			
	Adenosine (3.75 μM)	Aspirin (11 μM)	Aspirin (111 μM)	
Isomucronustyrene (**2**)	>1000	7.7	20	9.2
Hydroxyobtustyrene (**3**)	>1000	52	370	2.8
Formononetin (**4**)	>1000	190	>1000	>500
(±)-Mucronulatol (**5**)	>1000	130	>1000	63
(±)-Vestitol (**6**)	1000	92	1000	47
(±)-Medicarpin (**9**)	>1000	370	>1000	>500
(−)-Methylnissolin (**10**)	>1000	170	>1000	>500
Methyl 2-hydroxy-3,4-dimethoxybenzoate (**15**)	>1000	3.5	47	23
(−)-Isomucronuratol (**19**)	>1000	26	>1000	150

Each figure indicates the concentration (μM) of test material, which gave same degree of inhibitory effects as positive control. *a*) PG synthetase.

induced by AA and collagen. As it is observed in many cases, the inhibitory effects of extracts are not attributable to a single compound, but the effects are the sum of the activities of many compounds contained in medicinal plants.

Experimental

All melting points were determined on a Yanagimoto melting point apparatus and were uncorrected. Specific rotation was measured on a JASCO DIPO-140 digital polarimeter. ORD spectra was taken on a JASCO J-20 spectrometer. Ultraviolet (UV) spectra were taken on a Hitachi spectrophotometer, model 100-60. Infrared (IR) spectra were recorded on a JASCO DS-701G spectrometer. MS were measured on a JEOL JMS-DX-300 spectrometer. ^{1}H-NMR spectra were measured on a JEOL FX-100 spectrometer with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). All materials, unless otherwise specified, were of reagent grade or the highest grade available from commercial sources. The root heartwood of *D. odorifera* was purchased in the Taipei market. The method of the assay of PG synthetase (radioisotope method) was described in a previous paper.[16] The assay method of platelet aggregation was carried out as described in previous papers.[3g,17]

Preliminary Assay for the Extract of *D. odorifera* Dried heartwood (10 g) of *D. odorifera* was successively extracted with hexane, $CHCl_3$, MeOH and water. The inhibitory effects of these extracts to PG synthetase were 97, 99, 99 and 70%, respectively, at 150 μg/ml.

Isolation of Active Principles from *D. odorifera* In a large scale extraction, dried heartwood (2 kg) was extracted with MeOH and then the MeOH extract was fractionated into hexane- $CHCl_3$- and water-soluble fractions. The $CHCl_3$ fraction was further separated into C_6H_6 soluble and C_6H_6 insoluble fractions. Of those fractions the C_6H_6 soluble fraction showed the most potent inhibitory effect (96% at 150 μg/μl and 60% at 20 μg/μl). Therefore, the C_6H_6 soluble fraction was further separated as shown in Charts 1 and 2 to afford compounds **1**—**3**, **5**, **6**, **8**, **14** and **15**, as active principles. In the course of separation of the active compounds, this fraction also afforded compounds **4**, **7** and **9**—**13**, which had no significant effects on PG synthetase.

Obtustyrene (**1**) was obtained as a colorless solid and gave the following spectral data. HR MS: $C_{16}H_{16}O_2$ (M^+: *m/z* 240.1153, Calcd: 240.1151). MS *m/z* (rel. int. %): 240 (M^+, 100), 239 (28), 225 (21), 209 (28), 137 (22), 115 (46), 91 (32). IR ν_{max}^{Nujol} cm^{-1}: 3400, 3030, 2930, 1610, 1598, 1508, 1502. ^{1}H-NMR ($CDCl_3$) δ: 3.40 (2H, m, CH_2), 3.75 (3H, s, OCH_3), 4.56 (1H, s, OH), 6.08—6.44 (4H, m, CH=CH, C3-H, C5-H), 6.91 (1H, d, *J*=8.0 Hz, C6-H), 7.04—7.30 (5H, m, aromat.).

Isomucronustyrene (**2**) was obtained as colorless oil and gave the following spectral data. HR MS: $C_{17}H_{18}O_3$ (M^+: *m/z* 270.1285, Calcd: 270.1255). MS *m/z* (rel. int. %): 270 (M^+, 100), 255 (16), 239 (24), 167 (12), 115 (35), 91 (33). IR ν_{max}^{cap} cm^{-1}: 3510, 3430, 3030, 2940, 1615, 1598, 1492. ^{1}H-NMR ($CDCl_3$) δ: 3.48 (2H, m, CH_2), 3.85, 3.88 (each 3H, s, OCH_3), 5.52 (1H, s, OH), 6.32 (2H, m, CH=CH), 6.52 (1H, d, *J*=8.0 Hz, C4-H), 6.64 (1H, d, *J*=8.0 Hz, C5-H), 7.04—7.30 (5H, m, aromat.).

Hydroxyobtustyrene (**3**) was obtained as colorless plates from ether and hexane (mp 76—76.5 °C), and gave the following spectral data. HR MS: $C_{16}H_{16}O_3$ (M^+: *m/z* 256.1137, Calcd: 256.1100). MS *m/z* (rel. int. %): 256 (M^+, 100), 241 (12), 225 (15), 152 (78), 115 (41), 91 (49). IR ν_{max}^{KBr} cm^{-1}: 3400, 3180, 1600, 1509, 1488. ^{1}H-NMR (acetone-d_6) δ: 3.44 (2H, m, CH_2), 3.79 (3H, s, OCH_3), 6.36 (2H, m, CH=CH), 6.50 (2H, s, C5-H, C6-H), 7.00—7.40 (5H, m, aromat.), 7.40, 7.69 (each 1H, s, OH). ^{1}H-NMR (C_6D_6) δ: 3.31 (3H, s, OCH_3), 3.37 (2H, d, *J*=5.4 Hz, CH_2), 4.91, 4.97 (each 1H, s, OH), 6.29 (2H, m, CH=CH), 6.54 (1H, d, *J*=8.4 Hz, C6-H), 6.69 (1H, d, *J*=8.4 Hz, C5-H), 6.93—7.30 (5H, m, aromat.).

Formononetin (**4**) was obtained as pail brown needles from MeOH (mp 260—261 °C) and gave the following spectral data. MS *m/z* (rel. int. %): 268 (M^+, 100), 253 (16), 149 (19), 133 (11), 132 (75), 117 (16). IR ν_{max}^{KBr} cm^{-1}: 3120, 1635, 1618, 1590, 1565, 1510, 1450. ^{1}H-NMR (DMSO-d_6) δ: 3.86 (3H, s, OCH_3), 6.86 (1H, d, *J*=2.3 Hz, C8-H), 6.95 (2H, d, *J*=9.1 Hz, C3′-H, C5′-H), 6.98 (1H, dd, *J*=8.6, 2.3 Hz, C6′-H), 7.94 (2H, d, *J*=9.1 Hz, C2′-H, C6′-H), 7.94 (1H, d, *J*=8.6 Hz, C5-H), 8.28 (1H, s, C2-H).

(±)-Mucronulatol (**5**) was obtained as colorless prisms from MeOH (mp 225.5—226.5 °C) and gave the following spectral data. HR MS: $C_{17}H_{18}O_5$ (M^+: *m/z* 302.1134, Calcd: 302.1151). MS *m/z* (rel. int. %): 302 (M^+, 65), 180 (100), 168 (31), 167 (31), 165 (28), 135 (24), 133 (31), 123 (15), 107 (19). IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1616, 1590, 1498, 1438. ^{1}H-NMR (DMSO-d_6) δ: 2.55—4.20 (5H, m, C2-H_2, C3-H, C4-H_2), 3.75, 3.76 (each 3H, s, OCH_3), 6.14 (1H, d, *J*=2.0 Hz, C8-H), 6.21 (1H, dd, *J*=8.0, 2.0 Hz, C6-H), 6.50, 6.63 (each 1H, d, *J*=7.6 Hz, C5′-H, C6′-H), 6.79 (1H, d, *J*=8.0 Hz, C5-H), 8.52, 9.04 (each 1H, s, OH). **5** (30 mg) was acetylated with acetic anhydride (2 ml) and pyridine (1.5 ml) in the usual manner to afford a diacetate (21 mg). Colorless needles from MeOH (mp 134—134.5 °C); MS *m/z* (rel. int. %): 386 (M^+, 19), 344 (42), 302 (39), 180 (100), 168 (87), 167 (25).

(±)-Vestitol (**6**) was obtained as colorless microcrystals from $CHCl_3$ and hexane (mp 175.5—176 °C) and gave the following spectral data. MS *m/z* (rel. int. %): 272 (M^+, 35), 151 (11), 150 (100), 138 (17), 137 (33), 135 (14), 123 (11). IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1613, 1595, 1508, 1450, 1430. ^{1}H-NMR (acetone-d_6) δ: 2.60—4.35 (5H, m, C2-H_2, C3-H, C4-H_2) 3.72 (3H, s, OCH_3), 6.20—6.50 (4H, m, C6-H, C8-H, C5′-H, C3′-H), 6.84 (1H, d, *J*=8.2 Hz, C5-H or C6′-H), 6.98 (1H, d, *J*=8.0 Hz, C5-H or C6′-H), 7.98, 8.40 (each 1H, s, OH). Compound **6** (36 mg) was acetylated with acetic anhydride (2 ml) and pyridine (1.5 ml) in the usual manner to afford a diacetate (25 mg). Colorless needles from Et_2O and hexane (mp 107—107.5 °C); MS *m/z* (rel. int. %): 356 (M^+, 23), 314 (30), 272 (25), 192 (20), 150 (100), 138 (31), 137 (39), 78 (48).

(+)-Duartin (**7**) was obtained as colorless oil and gave the following spectral data. Specific rotation: $[\alpha]_D^{25}$ +3.42° (*c*=1.11, $CHCl_3$). ORD (*c*=0.218, dioxane) $[\alpha]_D^{25}$ (nm): +73° (300), +229° (288), +266° (282), +248° (272), +358° (268), +55° (253), +412° (248), +211° (229). HR MS: $C_{18}H_{20}O_6$ (M^+: *m/z* 332.1258, Calcd: 332.1259). MS *m/z* (rel. int. %): 332 (M^+, 86), 180 (100), 168 (77), 167 (55), 165 (46), 164 (28), 153 (29), 133 (53). ^{1}H-NMR ($CDCl_3$) δ: 2.80—2.90 (2H, m, C4-H_2), 3.30—3.70 (1H, m, C3-H), 3.89, 3.90, 3.91 (each 3H, s, OCH_3), 4.00 (1H, t, *J*=10.0 Hz, C2-H (axial)), 4.39 (1H, m, C2-H (equatorial)), 5.60, 5.67 (each 1H, s, OH), 6.49 (1H, d, *J*=8.5 Hz, C6-H), 6.60, 6.61 (each 1H, s, C5′-H, C6′-H), 6.70 (1H, d, *J*=8.5 Hz, C5-H). ^{1}H-NMR (C_6D_6) δ: 2.71—2.81 (2H, m, C4-H_2), 3.16 (3H, s, C4′-OCH_3), 3.20—3.75 (1H, m, C3-H), 3.64, 3.66 (each 3H, s, C8-OCH_3, C2′-OCH_3), 3.88 (1H, t, *J*=10.0 Hz, C2-H (axial)), 4.30 (1H, m, C2-H (equatorial)), 5.34, 5.64 (each 1H, s, OH), 6.19 (1H, d, *J*=8.5 Hz, C5′-H), 6.38 (1H, d, *J*=8.5 Hz, C6′-H), 6.56 (1H, d, *J*=8.7 Hz, C5-H), 6.76 (1H, d, *J*=8.7 Hz, C6-H).

(±)-Isoduartin (**8**) was obtained as colorless oil and gave the following spectral data. HR MS: $C_{18}H_{20}O_6$ (M^+: *m/z* 332.1258, Calcd: 332.1259). MS *m/z* (rel. int. %): 332 (M^+, 63), 180 (100), 168 (45), 167 (79), 165 (32), 164 (24), 153 (31), 133 (45). ^{1}H-NMR ($CDCl_3$) δ: 2.90—3.05 (2H, m, C4-H_2), 3.30—3.75 (1H, m, C3-H), 3.85, 3.91, 3.92 (each 3H, s, OCH_3), 4.09 (1H, t, *J*=9.9 Hz, C2-H (axial)), 4.47 (1H, m, C2-H (equatorial)), 5.64, 6.00 (each 1H, s, OH), 6.44 (1H, d, *J*=8.6 Hz, C5′-H), 6.50 (1H, d, *J*=8.4 Hz, C6-H), 6.74 (1H, d, *J*=8.4 Hz, C5-H), 6.79 (1H, d, *J*=8.6 Hz, C6′-H). ^{1}H-NMR (C_6D_6) δ: 2.55—2.95 (2H, m, C4-H_2), 3.28 (3H, s, C4′-OCH_3), 3.00—3.80 (1H, m, C3-H), 3.51, 3.61 (each 3H, s, C8-OCH_3, C3′-OCH_3), 4.00 (1H, t, *J*=10.0 Hz, C2-H (axial)), 4.35 (1H, m, C2-H (equatorial)), 5.67, 5.88 (each 1H, s, OH), 6.10 (1H, d, *J*=8.6 Hz, C5′-H), 6.55 (1H, d, *J*=8.4 Hz, C5-H), 6.58 (1H, d, *J*=8.6 Hz, C6′-H), 6.75 (1H, d, *J*=8.4 Hz, C6-H).

(±)-Medicarpin (**9**) was obtained as colorless needles from acetone and water (mp 200—201 °C) and gave the following spectral data. MS *m/z* (rel. int. %): 270 (M^+, 100), 269 (45), 255 (27), 148 (20). IR ν_{max}^{KBr} cm^{-1}: 3360, 2920, 1615, 1590, 1490, 1465, 1448. ^{1}H-NMR (acetone-d_6) δ: 3.40—3.80 (2H, m, C6-H (axial), C6a-H), 3.70 (3H, s, OCH_3), 4.10—4.36 (1H, m, C6-H (equatorial)), 5.48 (d, J=5.2 Hz, C11a-H), 6.22—6.56 (4H, m, C2-H, C4-H, C8-H, C10-H), 7.15 (1H, d, J=8.0 Hz, C1-H), 7.24 (1H, d, J=8.1 Hz, C7-H), 8.44 (1H, s, OH).

(−)-Methylnissolin (**10**) was obtained as colorless needles from hexane and AcOEt (mp 180—181.5 °C) and gave the following spectral data. Specific rotation: $[\alpha]_D^{25}$ −219° (c=0.465, $CHCl_3$). HR MS: $C_{17}H_{16}O_5$ (M^+: *m/z* 300.0990, Calcd: 300.0995). MS *m/z* (rel. int. %): 300 (M^+, 100), 285 (32), 147 (15). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3595, 3300, 1620, 1495, 1465, 1270, 1160, 1120, 1085, 950. ^{1}H-NMR ($CDCl_3$) δ: 3.35—3.79 (2H, m, C6-H (axial) and C6a-H), 3.84, 3.94 (each 3H, s, OCH_3), 4.15—4.32 (1H, m, C6-H (equatorial)), 5.25 (1H, s, OH), 5.52 (1H, d, J=6.3 Hz, C11a-H), 6.42 (1H, d, J=2.4 Hz, C4-H), 6.46 (1H, d, J=8.1 Hz, C8-H), 6.55 (1H, dd, J=2.4, 8.4 Hz, C2-H), 6.89 (1H, d, J=8.1 Hz, C7-H), 7.42 (1H, d, J=8.4 Hz, C1-H). ^{1}H-NMR (C_6D_6) δ: 2.85—4.00 (3H, m, C6-H_2, C6a-H), 3.38, 3.86 (each 3H, s, OCH_3), 4.25 (1H, s, OH), 5.14 (1H, d, J=6.5 Hz, C11a-H), 6.22 (1H, d, J=7.7 Hz, C8-H), 6.28 (1H, dd, J=8.7, 2.0 Hz, C2-H), 6.33 (1H, d, J=2.0 Hz, C4-H), 6.50 (1H, d, J=7.7 Hz, C7-H), 7.28 (1H, d, J=8.7 Hz, C4-H).

(−)-Melilotocarpan C (**11**) was obtained as colorless plates from MeOH (mp 157.5—159 °C) and gave the following spectral data. Specific rotation: $[\alpha]_D^{25}$ −170° (c=0.213, dioxane). HR MS: $C_{18}H_{18}O_6$ (M^+: *m/z* 330.1146, Calcd: 330.1102). MS *m/z* (rel. int. %): 330 (M^+, 100), 315 (38), 178 (12). ^{1}H-NMR ($CDCl_3$) δ: 3.40—3.90 (2H, m, C6-H (axial), C6a-H), 3.85, 3.92, 3.94 (each 3H, s, OCH_3), 4.20—4.45 (1H, m, C6-H (equatorial)), 5.48 (1H, s, OH), 5.58 (1H, d, J=6.0 Hz, C11a-H), 6.46 (1H, d, J=8.0 Hz, C8-H), 6.67 (1H, J=8.5 Hz, C2-H), 6.90 (1H, d, J=8.0 Hz, C7-H), 7.13 (1H, d, J=8.5 Hz, C1-H).

(−)-Melilotocarpan D (**12**) was obtained as colorless prisms from ethanol (mp 165—165.5 °C) and gave the following spectral data. Specific rotation: $[\alpha]_D^{25}$ −190° (c=0.120, dioxane). MS *m/z* (rel. int. %): 316 (M^+, 100), 301 (51). IR ν_{max}^{KBr} cm^{-1}: 3500, 3370, 1620, 1590, 1495, 1470, 1370. ^{1}H-NMR ($CDCl_3$) δ: 3.50—3.90 (2H, m, C6-H (axial), C6a-H), 3.88, 3.91 (each 3H, s, OCH_3), 4.20—4.45 (1H, m, C6-H (equatorial)), 5.34, 5.48 (each 1H, s, OH), 5.60 (1H, d, C11a-H), 6.46 (1H, d, J=8.3 Hz, C8-H), 6.66 (1H, d, J=8.7 Hz, C2-H), 6.77 (1H, d, J=8.3 Hz, C7-H), 7.15 (1H, d, J=8.7 Hz, C1-H).

(−)-Odoricarpan (**13**) was obtained as a colorless amorphous solid and gave the following spectral data. Specific rotation: $[\alpha]_D^{25}$ −136° (c=0.245, $CHCl_3$). ORD (c=0.018, dioxane) $[\alpha]_D^{25}$ (nm): −890° (295), −440° (286), −2110° (280), −4890° (249), −5330° (234), −7560° (222). HR MS: $C_{18}H_{18}O_6$ (M^+: *m/z* 330.1087, Calcd: 330.1102), MS *m/z* (rel. int. %): 330 (M^+, 100), 315 (67). ^{1}H-NMR ($CDCl_3$) δ: 3.40—3.95 (2H, m, C6-H (axial), C6a-H), 3.86, 3.88, 3.89 (each 3H, s, OCH_3), 4.15—4.45 (1H, m, C6-H (equatorial)), 5.32 (1H, s, OH), 5.58 (1H, d, J=6.0 Hz, C11a-H), 6.48 (1H, d, J=8.1 Hz, C8-H), 6.67 (1H, d, J=8.7 Hz, C2-H), 6.77 (1H, d, J=8.1 Hz, C7-H), 7.34 (1H, d, J=8.7 Hz, C1-H). ^{1}H-NMR (C_6D_6) δ: 2.85—4.05 (3H, m, C6-H_2, C6a-H), 3.16, 3.33 (each 3H, s, C3-OCH_3, C9-OCH_3), 3.83 (3H, s, C4-OCH_3), 5.11 (1H, s, OH), 5.27 (1H, d, J=6.5 Hz, C11a-H), 6.08 (1H, d, J=7.7 Hz, C2-H or C8-H), 6.33 (1H, d, J=8.7 Hz, C2-H or C8-H), 6.35 (1H, d, J=8.7 Hz, C1-H or C7-H), 6.35 (1H, d, J=7.7 Hz, C1-H or C7-H).

Odoriflavene (**14**) was obtained as colorless prisms from hexane and AcOEt (mp 177.5—179 °C) and gave the following spectral data. HR MS: $C_{17}H_{16}O_5$ (M^+: *m/z* 300.1008, Calcd: 300.0998). MS *m/z* (rel. int. %): 300 (M^+, 100), 299 (29), 285 (27), 180 (20), 150 (14), 147 (11), 143 (17), 135 (14). IR ν_{max}^{KBr} cm^{-1}: 3245, 1610, 1495, 1460, 1430. ^{1}H-NMR (acetone-d_6) δ: 3.80 (3H, s, C3′-OCH_3), 3.86 (3H, s, C4′-OCH_3), 5.02 (2H, d, J=1.3 Hz, CH_2), 6.32 (1H, d, J=2.4 Hz, C8-H), 6.41 (1H, dd, J=2.4, 7.9 Hz, C6-H), 6.57 (1H, d, J=8.8 Hz, C5′-H), 6.69 (1H, br s, −CH=), 6.95 (1H, d, J=7.9 Hz, C5-H), 7.00 (1H, d, J=8.8 Hz, C6′-H), 8.10, 8.45 (each 1H, s, OH).

Methyl 2-hydroxy-3,4-dimethoxybenzoate (**15**) was obtained as colorless needles from butanol (mp 86—87.5 °C) and gave the following spectral data. MS *m/z* (rel. int. %): 212 (M^+, 26), 181 (43), 180 (31), 151 (72), 137 (100). IR ν_{max}^{KBr} cm^{-1}: 3300, 1710, 1605, 1495, 1430, 1290, 1215. ^{1}H-NMR ($CDCl_3$) δ: 3.90 (3H, s, $COOCH_3$), 3.94 (6H, s, C3-OCH_3, C4-OCH_3), 5.73 (1H, s, OH), 6.69 (1H, d, J=9.0 Hz, C5-H), 7.46 (1H, d, J=9.0 Hz, C6-H).

Synthesis of Obtustyrene (1) Ethanolic solution (0.3 ml) of 4-hydroxy-2-methoxyacetophenone (81.6 mg) and acetophenone (65 mg), and aqueous KOH (2.5 g in 2 ml) was stirred at room temperature until the appearance of an amorphous solid. Then, the mixture was acidified by HCl and extracted with Et_2O. After evaporating to dryness, the extract was purified with a silica gel column and recrystallized to afford the corresponding chalcone (69 mg). A solution of the chalcone (32 mg) in dioxane (5 ml) was added dropwise to an Et_2O solution containing $LiAlH_4$ (28.5 mg) and $AlCl_3$ (315 mg). After decomposition of excess reagent by aqueous EtOH, the mixture was acidified with HCl and extracted with Et_2O. Evaporation of Et_2O followed by silica gel column purification afforded **1** (15 mg).

Synthesis of Isomucronustyrene (2) 2,6-Dimethoxyphenol (770 mg) and cinnamylalcohol (335 mg) in 75% formic acid were stirred at 60 °C for 30 min. After evaporating the solvent, the residual was dissolved in Et_2O and washed with $NaHCO_3$ and NaCl solutions. Then, the Et_2O fraction was evaporated and purified with repeated silica gel column chromatographies to afford **2** (52 mg).

Synthesis of Hydroxyobtustyrene (3) and Compounds 17 and 18 *o*-Methoxycatechol (700 mg) and cinnamylalcohol (670 mg) in 75% formic acid (20 ml) were stirred at 60 °C for 30 min. After adding water (20 ml), the aqueous solution was extracted with C_6H_6. The extract was washed with NaCl solution, followed by evaporation to dryness. The residual was fractionated with silica gel, Lobar Si-60 and Sephadex LH-20 column chromatographies to afford **3** (61 mg), **17** (5 mg) and **18** (11 mg). **17**: colorless oil; MS *m/z* (rel. int. %): 256 (M^+, 86), 152 (100), 115 (18), 91 (52); ^{1}H-NMR (C_6D_6) δ: 3.19 (3H, s, OCH_3), 3.60 (2H, d, J=4.9 Hz, CH_2), 5.13 (2H, br s, OH), 6.14 (1H, d, J=8.6 Hz, C5-H), 6.43 (2H, m, CH=CH), 6.67 (1H, d, J=8.6 Hz, C6-H), 6.98—7.32 (5H, m, aromat.). **18**: colorless oil; MS *m/z* (rel. int. %): 372 (M^+, 74), 268 (50), 256 (100), 255 (45), 152 (73), 149 (38), 117 (53), 115 (49), 91 (95). ^{1}H-NMR (C_6D_6) δ: 3.34 (3H, s, OCH_3), 3.41 (2H, d, J=5.1 Hz, CH_2), 3.53 (2H, d, J=4.8 Hz, CH_2), 5.15 (2H, br s, OH), 6.25—6.45 (4H, m, CH=CH), 6.66 (1H, d, J=8.6 Hz, C5-H), 6.95—7.30 (10H, m, aromat.). IC_{50} values of compounds **17** and **18** against PG synthetase were 5.9 and 27 μM, respectively.

Catalytic Reduction of (−)-Methylnissolin (10) and Odoriflavene (14) (−)-Methylnissolin (**10**; 100 mg) in AcOH (3 ml) was catalytically reduced under H_2 over 10% Pd/C (50 mg). After filtrating the catalysts, the solution was evaporated to dryness. The residual was purified by a silica gel column chromatography and recrystallization from AcOEt and hexane to afford (−)-isomucronulatol (**19**; 71 mg). **19**: colorless needles (mp 149.5—150 °C); specific rotation: $[\alpha]_D^{25}$ −7.5° (c=0.567, acetone); MS *m/z* (rel. int. %): 302 (M^+, 55), 180 (100), 168 (40), 167 (44), 165 (13), 151 (12), 135 (13), 133 (16); ^{1}H-NMR (acetone-d_6) δ: 2.60—3.15 (2H, m, C4-H_2), 3.30—3.60 (1H, m, C3-H), 3.79, 3.82 (each 3H, s, OCH_3), 3.99 (1H, t, J=9.8 Hz, C2-H (axial)), 4.26 (1H, m, C2-H (equatorial)), 6.28 (1H, d, J=2.4 Hz, C8-H), 6.35 (1H, dd, J=2.4, 7.9 Hz, C6-H), 6.50 (1H, d, J=8.5 Hz, C5′-H), 6.84 (1H, d, J=8.5 Hz, C6′-H), 6.90 (1H, J=7.9 Hz, C5-H).

Odoriflaven (**14**; 5 mg) in MeOH (5.5 ml) was catalytically reduced under H_2 over 10% Pd/C (5 mg). The resulting mixture was worked up in the same manner as **10**. After chromatographic purification the obtained colorless oil gave the same MS and ^{1}H-NMR spectra as those of **19**.

References and Notes

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3) *a*) F. Kiuchi, M. Shibuya and U. Sankawa, *Chem. Pharm. Bull.*, **30**, 754 (1982); *b*) *Idem, ibid.*, **30**, 2279 (1982); *c*) X.-S. Yao, Y. Ebizuka, H. Noguchi, F. Kiuchi, U. Sankawa and H. Seto, *Tetrahedron Lett.*, **24**, 2407 (1983); *d*) *Idem, ibid.*, **24**, 3247 (1983); *e*) X.-S. Yao, Y. Ebizuka, H. Noguchi, F. Kiuchi, H. Seto and U. Sankawa, *ibid.*, **25**, 5541 (1984); *f*) C.-F. Tseng, A. Mikajiri, M. Shibuya, Y. Goda, Y. Ebizuka, K. Padwaminata and U Sankawa, *Chem. Pharm. Bull.*, **34**, 1380 (1986); *g*) Y. Goda, M. Shibuya and U. Sankawa, *ibid.*, **35**, 2668 (1987); *h*) *Idem, ibid.*, **35**, 2675 (1987); *i*) X.-S. Yao, Y. Ebizuka, H. Noguchi, F. Kiuchi, M. Shibuya, Y. Iitaka, H. Seto and U. Sankawa, *ibid.*, **39**, 2956 (1991); *j*) *Idem, ibid.*, **39**, 2962 (1991).
4) J. R. Vane, *Nature New Biology*, **231**, 232 (1971).
5) *a*) A. Hirai and Y. Tamura, *Thrombosis Research*, **31**, 29 (1983); *b*) Y. Koshihara, T. Neichi, S. Murota, A.-N. Lao, Y. Fujimoto and T. Tatsuno, *FEBS Lett.*, **158**, 41 (1983); *c*) Y. Goda, M. Katayama, K. Ichikawa, M. Shibuya, F. Kiuchi and U. Sankawa, *Chem. Pharm. Bull.*, **33**, 5606 (1985).
6) "Encyclopedia of Chinese Materia Medica (Zhon Yao Dai Zi Ten)," ed. by Jiang Su New Medical College, Shanghai Science and Technology Publisher, Shanghai, 1977, pp. 1475—1476.

7) Y. Goda and U. Sankawa, unpublished observation.
8) *a*) S. Mageswaran, W. D. Ollis, R. J. Roberts and I. O. Sutherland, *Tetrahedron Lett.*, **34**, 2897 (1969); *b*) M. Gregson, W. D. Ollis, B. T. Redman, I. O. Sutherland, H. H. Dietrichs and O. R. Gottlieb, *Phytochemistry*, **17**, 1395 (1978).
9) K. Kurosawa, W. D. Ollis, I. O. Sutherland, O. R. Gottlieb and A. B. de Oliveira, *Phytochemistry*, **17**, 1405 (1978).
10) K. Kurosawa, W. D. Ollis, B. T. Redman, I. O. Sutherland and O. R. Gottlieb, *Phytochemistry*, **17**, 1413 (1978).
11) D. J. Robeson and J. L. Ingham, *Phytochemistry*, **18**, 1715 (1979).
12) T. Miyase, A. Ohtsuboi, A. Ueno, T. Noro, M. Kuroyanagi and S. Fukushima, *Chem. Pharm. Bull.*, **30**, 1986 (1982).
13) Authentic formation, (±)-vesitol and (±)-medicarpin were kind gifts from Dr. T. Kinoshita in our faculty. Authentic melilotocarpan C and D were kindly supplied by Prof. S. Fukushima (Shizuoka College of Pharmacy).
14) A. Pelter and P. I. Amenchin, *J. Chem. Soc.* (*C*), **1969**, 887; R. G. Wilson, J. H. Bowie and D. H. Williams, *Tetrahedron*, **24**, 1407 (1968).
15) W. D. Ollis, I. O. Sutherland, H. M. Alves and O. R. Gottlieb, *Phytochemistry*, **17**, 1401 (1978).
16) F. Kiuchi, M. Shibuya and U. Sankawa, *Chem. Pharm. Bull.*, **31**, 3391 (1983).
17) S. Iwakami, J.-B. Wu, Y. Ebizuka and U. Sankawa, *Chem. Pharm. Bull.*, **40**, 1196 (1992).