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Studies on Coumarins from the Root of *Angelica pubescens* Maxim. III.[1] Structures of Various Coumarins including Angelin, a New Prenylcoumarin

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Two prenylcoumarins, XII and XIII, were isolated from the root of *Angelica pubescens* Maxim. (Umbelliferae) growing in Japan, together with eleven known coumarins, osthol(I), bergapten(II), glabra-lactone(III), angelol(IV), psoralen(V), xanthotoxin(VI), isopimpinellin(VII), byak-angelicin(VIII), coumurrayin(IX), 7-methoxy-8-senecioylcoumarin(X) and scopoletin(XI). The structures of XII and XIII were elucidated as 8-(3-hydroxyisovaleroyl)-5,7-dimethoxycoumarin and 5-isopentenyloxy-7-methoxy-8-senecioylcoumarin, respectively, by chemical and spectral studies. XIII is a new natural prenylcoumarin, and was named angelin.

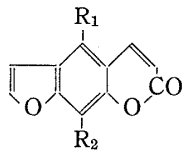
Keywords——*Angelica pubescens* Maxim.; Umbelliferae; coumarins; prenylcoumarins; angelin; 5-isopentenyloxy-7-methoxy-8-senecioylcoumarin; 8-(3-hydroxyisovaleroyl)-5,7-dimethoxycoumarin

The dried roots of *Angelica pubescens* Maxim. (Umbelliferae; Japanese name, shishiudo) have been used as a crude drug, "maki-kyokatsu" or "naga-kyokatsu", in Japan. On the other hand, in China a plant with the same botanical name is called "mao-danggui" and its dried root has been used as the Chinese crude drug "duhuo" or "xiang-duhuo". However, there is difference between the Chinese crude drug "duhuo", whose plant of origin is thought to be *Angelica pubescens* Maxim., and the dried root of Japanese "shishiudo" as regards the content of coumarin compounds. This difference seems to arise from the confusion of different plants, since the root of *Angelica pubescens* Maxim. forma *biserrata* Shan *et* Yuan "chongchi-mao-danggui" and *Angelica porphyrocaulis* Nakai *et* Kitag. "zijing-duhuo", *etc.*, have been used as the Chinese crude drug "duhuo". However, even though different plants are used, they appear to be of the same genus, *Angelica*, and should be rather closely related plant species. This problem is now under investigation.

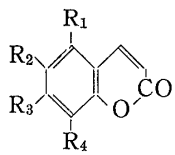
The authors have already reported that the roots of this plant grown in Japan afforded four coumarins, osthol (I), bergapten (II), glabra-lactone (III) and angelol (IV).[3] Recently, for comparison with the Chinese crude drug "duhuo", the authors have reinvestigated these roots more closely and isolated nine coumarins, psoralen (V), xanthotoxin (VI), isopimpinellin (VII), byak-angelicin (VIII), coumurrayin (IX), 7-methoxy-8-senecioylcoumarin (X), scopoletin (XI) and the prenylcoumarin derivatives XII and XIII in addition to the above four coumarins. This paper deals with the structure elucidation of compounds XII and XIII.

The dried roots of *Angelica pubescens* Maxim. were treated as described in the experimental section and separated into hexane, ethyl acetate, acetone and methanol extracts. The compounds in question were isolated from the ethyl acetate extract by silica gel column chromatography and preparative thin-layer chromatography. Compounds I—XI were identified by comparison with authentic samples, but on examination of the cold methanol extract of the same material by preparative thin-layer chromatography it was confirmed that byak-

1) Part II: K. Hata and M. Kozawa, *Yakugaku Zasshi*, **88**, 293 (1968).
2) Location: *Kawai 2-chome, Matsubara, Osaka.*
3) K. Hata and M. Kozawa, *Yakugaku Zasshi*, **88**, 283 (1968).



II: $R_1=OCH_3$, $R_2=H$
V: $R_1=R_2=H$
VI: $R_1=H$, $R_2=OCH_3$
VII: $R_1=R_2=OCH_3$
VIII: $R_1=OCH_3$, $R_2=OCH_2-CH(OH)-C(OH)(CH_3)(CH_3)$
XIV: $R_1=OCH_3$, $R_2=OCH_2-CH(O\diagup\diagdown)-C(CH_3)(CH_3)$



I: $R_1=R_2=H$, $R_3=OCH_3$, $R_4=CH_2-CH=C(CH_3)(CH_3)$
III: $R_1=R_3=OCH_3$, $R_2=H$, $R_4=CO-CH=C(CH_3)(CH_3)$
IV: $R_1=R_4=H$, $R_3=OCH_3$, $R_2=CH(O\text{-angeloyl})-CH(OH)-C(OH)(CH_3)(CH_3)$
IX: $R_1=R_3=OCH_3$, $R_2=H$, $R_4=CH_2-CH=C(CH_3)(CH_3)$
X: $R_1=R_2=H$, $R_3=OCH_3$, $R_4=CO-CH=C(CH_3)(CH_3)$
XI: $R_1=R_4=H$, $R_2=OCH_3$, $R_3=OH$

Chart 1

angelicin was an artifact which was formed by cleavage of the epoxy ring in ferulin (XIV) upon reflux extraction or silica gel column chromatography.

Compound XII, mp 180—182° (dec.), white needles, $C_{16}H_{18}O_6$ showed a blue fluorescence under filtered ultraviolet light on a thin-layer chromatogram and showed infrared (IR) absorption bands due to a hydroxyl group and coumarin ring. The proton magnetic resonance (PMR) spectrum (δ ppm, $CDCl_3$) of XII exhibited signals arising from the 3-hydroxy isovaleryl group at 1.35 (6H, s), 3.02 (2H, s) and 3.85 (1H, br. s), two methoxyl groups at 3.88 (3H, s), 3.93 (3H, s), and two protons at the 3- and 4-positions of the coumarin ring at 6.10 (1H, d, J=9.5 Hz), 7.88 (1H, d, J=9.5 Hz), as well as a signal attributable to a phloroglucinol-type benzene or aromatic proton at 6.27 (1H, s) (shifted to higher field). These data indicate that XII is a 5,7-dimethoxycoumarin derivative. XII was decomposed upon heating at 170—180° under 0.05 mm/Hg to afford white crystals (XV), mp 192—193°, showing a pale blue fluorescence under ultraviolet light on a silica gel thin-layer chromatogram. The PMR spectrum of XV showed signals assignable to a benzene or an aromatic proton, two methoxyl groups, two protons at the 3-, 4-positions of the coumarin ring and an acetyl group which was assumed to be formed by cleavage of the 3-hydroxyisovaleroly chain as a result of the heating. Based on this PMR spectrum and its melting point, XV appeared to be identical with 5,7-dimethoxy-8-acetylcoumarin, which was obtained by alkali hydrolysis of glabra-lactone, and this was confirmed by direct comparison with an authentic sample prepared from glabra-lactone. Therefore, the side chain of XII must be at the 8-position and the structure of XII was established as 8-(3-hydroxyisovaleroyl)-5,7-dimethoxy-coumarin. This structure was confirmed by the following results. Heating of glabra-lactone with 5% oxalic acid gave its hydrate, which afforded not the acetate, but glabra-lactone upon heating with acetic anhydride and sodium acetate; XII gave glabra-lactone upon similar acetylation.

On the basis of these finding, XII was established to be 8-(3-hydroxyisovaleroyl)-5,7-dimethoxycoumarin. This compound has already been reported as a glabra-lactone derivative, but this is the first time that the compound has been obtained as a natural product.

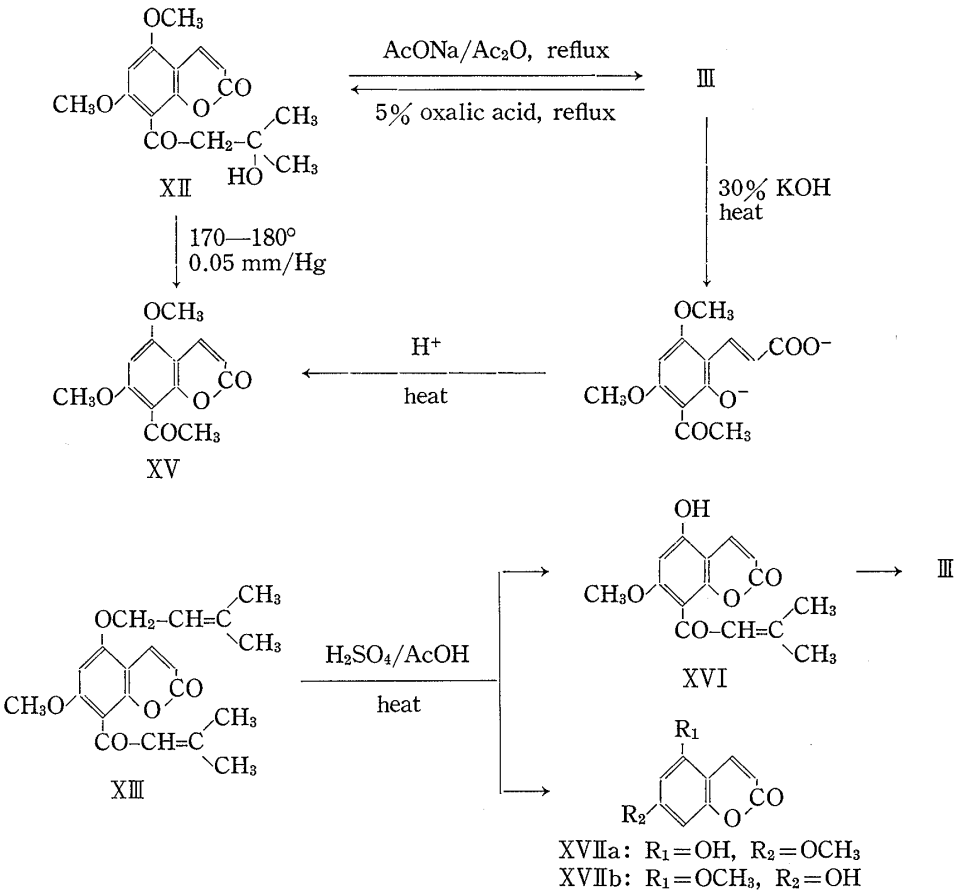


Chart 2

Compound XIII, mp 143—144°, white needles, $C_{20}H_{22}O_5$, showed IR absorption bands due to a hydroxyl group, carbonyl group and coumarin ring. The PMR spectrum (δ ppm, $CDCl_3$) of XIII showed signals at 1.77 (3H, d, J=1.0 Hz), 1.82 (2H, d, J=1.0 Hz), 4.62 (2H, d. J=6.0 Hz) and 5.47 (1H, m), which are assignable to an isopentenyloxy group, as well as signals assignable to a senecioyl group at 1.94 (3H, d, J=1.0 Hz), 2,33 (3H, d, J=1.0 Hz) and 6.31 (1H, m), and signals arising from a methoxyl group at 3.78 (3H, s), two protons at the 3- and 4-positions of the coumarin ring at 6.10 (1H, d, J=9.5 Hz), 7.96 (1H, J=9.5 Hz) and a benzene or an aromatic proton at 6.31 (1H, s), which suggested a phloroglucinol-type compound similar to XII. XIII was decomposed upon heating in acetic acid with a small quantity of sulfuric acid to afford two compounds, XVI, mp 205—206° and XVII, mp 226°. The PMR spectrum of XVI exhibited signals (δ ppm, acetone-d_6) attributable to a senecioyl group, a methoxyl group and an unchelated phenolic hydroxyl group, but showed no isopentenyloxy group signals like those observed in XIII. Therefore, XVI can be assumed to be a phenolic compound formed by cleavage of the isopentenyloxy group of XIII. Furthermore, methylation of XVI with dimethyl sulfate in acetone under reflux gave glabra-lactone (III). On the basis of these findings, the structure of XVI was suggested to be 5-hydroxy-7-methoxy-8-senecioylcoumarin.

The PMR spectrum of XVII showed the signals (δ ppm, CD_3OD) of a hydroxyl group, a methoxyl group, two mutually *meta*-situated protons on an aromatic ring and two protons at the 3- and 4-positions of the coumarin ring. These spectral data indicate that XVII is either 5-hydroxy-7-methoxycoumarin (XVIIa) or 7-hydroxy-5-methoxycoumarin (XVIIb). However, both coumarins have been reported; the former melted at 228° and the latter at 246°. Consequently, XVII must be XVIIa because it gave mp 226° and showed a positive

Gibbs test. Further evidence for this assignment was obtained by comparison with authentic samples of XVIIa and XVIIb.

From the results described above, the structure of XIII was established to be 5-isopentenyloxy-7-methoxy-8-senecioylcoumarin. This is a new natural prenylcoumarin, and was named angelin.

The authors have now isolated thirteen coumarin derivatives from the roots of *Angelica pubescens* Maxim. growing in Japan and determined their structures. However, the main coumarin compounds of this plant root are angelol-type prenylcoumarins, and the authors have been found several coumarins of this type. Isolation and structure elucidation of these coumarins are now in progress and the results will be reported in a subsequent paper.

Experimental

Apparatus——IR: Hitachi EPI-G2, PMR: Hitachi R-24A, R-40, melting point: Buchi melting point measuring apparatus.

Isolation of the Compounds——The dried and crushed roots (8 kg) of the plant collected in Nara Pref. during May, 1978 were extracted 3 times by refluxing with 15 liters each of hexane, then ethyl acetate, acetone and methanol for 5 hr (for each extraction). Each solution was concentrated under reduced pressure to give the corresponding extract: hexane extract (173.8 g), ethyl acetate extract (215 g), acetone extract (110.6 g) and methanol extract (68 g). The hexane extract was further treated with hexane at room temperature and divided into soluble (77.5 g) and insoluble parts (96.3 g). The insoluble part was added to the ethyl acetate extract, then this was chromatographed on silica gel (2.0 kg) with a mixture of hexane and ethyl acetate and divided into several fractions containing coumarins, which yielded I—XIII upon re-chromatography using silica gel. In addition, the dried roots (100 g) were separately extracted 3 times with 300 ml each of methanol at room temperature for 12 hr (for each fraction), and the methanol extract was separated by preparative TLC (silica gel) to give XIV.

Osthol (I)——Recrystallized from hexane–EtOAc to give white needles. mp 81—82°. The melting point showed no depression on admixture with an authentic sample of osthol. The IR and PMR spectra were identical with those of the authentic sample. Yield 0.01%.

Bergapten (II)——Recrystallized from hexane–EtOAc to give white needles. mp 184—185°. The melting point showed no depression on admixture with an authentic sample of bergapten. The IR and PMR spectra were identical with those of the authentic sample. Yield 0.003%.

Glabra-lactone (III)——Recrystallized from hexane–EtOAc to give white needles. mp 128—129°. The melting point showed no depression on admixture with an authentic sample of glabra-lactone. The IR and PMR spectra were identical with those of the authentic sample. Yield 0.19%.

Angelol (IV)——Recrystallized from ethyl ether to give white needles. mp 104—105°. $[\alpha]_D^{24}$ −93.5° (c=1.1, $CHCl_3$). The IR and PMR spectra were identical with those of the authentic sample of angelol. Yield 0.0009%. (Yield of total angelol-type coumarins, 2.3%).

Psoralen (V)——Recrystallized from hexane–EtOAc to give a white crystalline powder. mp 159—160°. The melting point showed no depression on admixture with an authentic sample of psoralen. The IR and PMR spectra were identical with those of the authentic sample. Yield 0.0019%.

Xanthotoxin (VI)——Recrystallized from hexane–EtOAc to give white needles. mp 143—144°. The melting point showed no depression on admixture with an authentic sample of xanthotoxin. The IR and PMR spectra were identical with those of the authentic sample. Yield 0.0049%.

Isopimpinellin (VII)——Recrystallized from hexane–EtOAc to give pale yellow needles. mp 147—148°. The melting point showed no depression on admixture with an authentic sample of isopimpinellin. The IR and PMR spectra were identical with those of the authentic sample. Yield 0.0018%.

Byak-angelicin (VIII)——Recrystallized from EtOAc to give pale yellow needles. mp 115—116°. The melting point showed no depression on admixture with an authentic sample of byak-angelicin. The IR and PMR spectra were identical with those of the authentic sample. Yield 0.0004%.

Ferulin (XIV)——Recrystallized from hexane–EtOAc to give pale yellow needles. mp 83—84°. $[\alpha]_D^{28}$ +9.83° (c=1.2, pyridine). The IR and PMR spectra were identical with those of an authentic sample of ferulin. Yield 0.0025%.

Coumurrayin (IX)——Recrystallized from hexane–EtOAc to give white needles. mp 154—155° (lit. mp 158°). *Anal.* Calcd for $C_{16}H_{18}O_4$: C, 70.06; H, 6.61. Found: C, 70.29; H, 6.49. The IR and PMR spectra were identical with those of an authentic sample of coumurrayin. Yield 0.0007%.

7-Methoxy-8-senecioylcoumarin (X)——Recrystallized from hexane–EtOAc to give a white crystalline powder. mp 128.5—129°. The melting point showed no depression on admixture with an authentic sample of 7-methoxy-8-senecioylcoumarin. The IR and PMR spectra were identical with those of the authentic sample. Yield 0.051%.

Scopoletin (XI)——Recrystallized from EtOAc to give a white crystalline powder. mp 204—205°. The melting point showed no depression on admixture with an authentic sample of scopoletin. The IR and PMR spectra were identical with those of the authentic sample. Yield 0.0021%.

8-(3-Hydroxyisovaleroyl)-5,7-dimethoxycoumarin (XII)——Recrystallized from hexane–EtOAc to give white needles, mp 180—182° (dec.). *Anal.* Calcd for $C_{16}H_{18}O_6$: C, 62.74; H, 5.92. Found: C, 62.63; H, 5.63. UV λ_{max}^{EtOH} nm (log ε): 207 (4.30), 218 (4.30), 259 (3.85), 321 (4.26). IR ν_{max}^{Nujol} cm^{-1}: 3540 (OH), 1720 (C=O), 1695 (C=O), 1620, 1600 (arom.). PMR (in $CDCl_3$) δ ppm:[4] 1.35 (6H, s), 3.02 (2H, s), 3.85 (1H, br. s), 3.88 (3H, s), 3.93 (3H, s), 6.27 (1H, s), 6.10 (1H, d, J=9.5 Hz), 7.88 (1H, d, J=9.5 Hz). Yield 0.005%.

Angelin (XIII)——Recrystallized from hexane–EtOAc to give white needles. mp 143—145°. *Anal.* Calcd for $C_{20}H_{22}O_5$: C, 70.20; H, 6.40. Found: C, 70.34; H, 6.21. UV λ_{max}^{EtOH} nm (log ε): 208 (4.47), 222 (4.19), 250 (4.30), 321 (4.28). IR ν_{max}^{Nujol} cm^{-1}: 1720 (C=O), 1660 (C=O), 1610, 1600 (arom.). PMR (in $CDCl_3$) δ ppm: 1.77 (3H, d, J=1.0 Hz), 1.82 (3H, d, J=1.0 Hz), 1.94 (3H, d, J=1.0 Hz), 2.33 (3H, d, J=1.0 Hz), 3.87 (3H, s), 4.62 (2H, d, J=6.0 Hz), 5.47 (1H, m), 6.31 (1H, s), 6.31 (1H, m), 6.10 (1H, d, J=9.5 Hz), 7.96 (1H, d, J=9.5 Hz). Yield 0.0058%.

Formation of XV from XII——Compound XII (20 mg) was heated at 170—180° under 0.05 mmHg to afford white needles (XV). mp 192—193°. The melting point showed no depression on admixture with an authentic sample of 5,7-dimethoxy-8-acetyl coumarin prepared from glabralactone. The IR and PMR spectra were identical with those of the authentic sample. Yield 8 mg.

Formation of XV from III——Compound III (1 g) was added to aq. 30% KOH (30 ml) and the mixture was heated under reflux for 30 min. The reaction mixture was diluted with H_2O (100 ml) and acidified with H_2SO_4, then a small amount of EtOH was added and the whole was heated on a boiling water bath for 1 hr. The solution was extracted with ethyl ether and the ether solution was washed with water, dried and evaporated to dryness. The residue was purified by chromatography on silica gel, using hexane–EtOAc as an eluent, and recrystallized to give white needles (XV), 520 mg, mp 192—193° (lit. 192—193°). IR ν_{max}^{Nujol} cm^{-1}: 1710 (C=O), 1680 (C=O), 1620, 1600 (arom.). PMR (in $CDCl_3$) δ ppm: 2.69 (3H, s), 3.98 (3H, s), 4.00 (3H, s), 6.37 (1H, s), 6.12 (1H, d, J=9.5 Hz), 7.92 (1H, d, J=9.5 Hz).

Formation of XII from III——Compound III (1 g) was added to aq. 5% oxalic acid and the mixture was heated under reflux for 15 hr. The reaction mixture was diluted with water (50 ml) and the precipitate was collected and purified by chromatography on silica gel, using hexane–EtOAc as an eluent, to give white needles (XII), 200 mg, mp 180—182°. The melting point showed no depression on admixture with XII. The IR and PMR spectra were identical with those of XII.

Formation of III from XII——Compound XII (100 mg) and NaOAc (200 mg) were added to Ac_2O (10 ml) and the mixture was heated under reflux for 1 hr, and treated in the usual way. The precipitate was collected and recrystallized from hexane–EtOAc to give white needles (III), mp 128—129°, 75 mg. The melting point showed no depression on admixture with an authentic sample of glabra-lactone (III). The IR and PMR spectra were identical with those of the authentic sample.

Cleavage of XIII with H_2SO_4/AcOH, Formation of XVI and XVII——Compound XIII (220 mg) was dissolved in AcOH (5 ml) and 5 drops of conc. H_2SO_4 were added. After stirring, the mixture was heated on a boiling water for 2 min then diluted with water (50 ml) and extracted with EtOAc. The EtOAc solution was washed with water, dried and concentrated *in vacuo*. The residue was purified by chromatography on silica gel, using hexane-EtOAc as an eluent, to afford XVI (20 mg) and XVII (15 mg).

XVI——Recrystallized from hexane–EtOAc to give a white crystalline powder, mp 205—206°. IR ν_{max}^{Nujol} cm^{-1}: 3300 (OH), 1685 (C=O), 1665 (C=O), 1600, 1565 (arom.). PMR (in acetone-d_6) δ ppm: 1.92 (3H, s), 2.16 (3H, s), 3.82 (3H, s), 6.28 (1H, m), 6.51 (1H, s), 6.08 (1H, d, J=9.5 Hz), 8.04 (1H, d, J=9.5 Hz), 9.78 (1H, br. s).

XVII——Recrystallized from hexane–EtOAc to give a white crystalline powder, mp 226°, Gibbs test: light blue color. The melting point showed no depression on admixture with an authentic sample of 5-hydroxy-7-methoxycoumarin (XVIIa). The IR and PMR spectra were identical with those of the authentic sample.

Methylation of XVI, Formation of III——Compound XVI (15 mg) was dissolved in dry acetone (10 ml), then 1 drop of dimethyl sulfate and K_2CO_3 (100 mg) were added and the mixture was heated under reflux for 1 hr. The reaction mixture was diluted with water (50 ml) and extracted with EtOAc. The EtOAc solution was washed with water, dried and concentrated *in vacuo*. The residue was recrystallized from hexane–EtOAc to afford III (14 mg), mp 128—129°. The melting point showed no depression on admixture with an authentic sample of glabra-lactone. The IR and PMR spectra were identical with those of the authentic sample.

Methylation of 5,7-Dihydroxycoumarin, Formation of XVIIa and XVIIb——5,7-Dihydroxycoumarin (280 mg) was dissolved in dry acetone (30 ml), then dimethyl sulfate (118 mg) and K_2CO_3 (500 mg) were added and the whole was heated under reflux for 1 hr. The reaction mixture was diluted with water (50 ml) and extracted with EtOAc, then the EtOAc solution was washed with water, dried and evaporated to dryness

4) The PMR spectra were measured using TMS as an internal standard.

in vacuo. The residue was purified by chromatography on silica gel, using hexane–EtOAc as an eluent, to give XVIIa (30 mg) and XVIIb (40 mg).

5-Hydroxy-7-methoxycoumarin (XVIIa)——Recrystallized from hexane–EtOAc to give a white crystalline powder, mp 226—228° (lit. mp 228—229°). Gibbs test: light blue color. IR ν_{max}^{Nujol} cm^{-1}: 3240 (OH), 1680 (C=O), 1620, 1600 (arom.). PMR (in CD_3OD) δ ppm: 3.82 (3H, s), 6.30 (1H, d, $J=2.0$ Hz), 6.38 (1H, d, $J=2.0$ Hz), 6.10 (1H, d, $J=9.5$ Hz), 8.07 (1H, d, $J=9.5$ Hz).

7-Hydroxy-5-methoxycoumarin (XVIIb)——Recrystallized from hexane–EtOAc to give a white crystalline powder, mp 242—244° (lit. 246°). Gibbs test: negative, IR ν_{max}^{Nujol} cm^{-1}: 3250 (OH), 1680 (C=O), 1620, 1600 (arom.). PMR (in CD_3OD) δ ppm: 3.88 (3H, s), 6.31 (1H, d, $J=2.0$ Hz), 6.34 (1H, d, $J=2.0$ Hz), 6.03 (1H, d, $J=9.5$ Hz), 7.98 (1H, d, $J=9.5$ Hz).

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