Structure and Synthesis of Kotanin and Desmethylkotanin, Metabolites of *Aspergillus glaucus*

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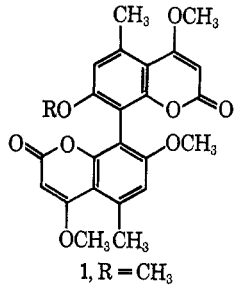
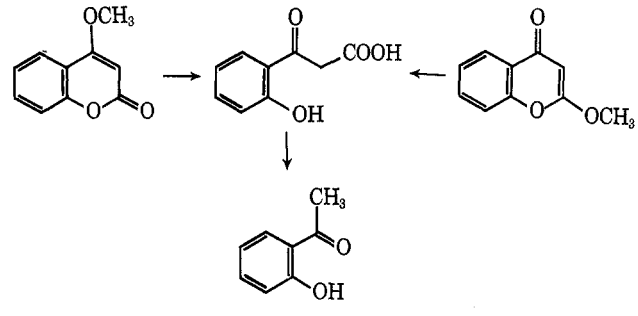
Two new metabolites, for which the names kotanin and desmethylkotanin are suggested, have been isolated from *Aspergillus glaucus* cultures. Spectral data on the metabolites and their base hydrolysis products were used to derive structures which were confirmed by total synthesis of racemic kotanin. Oxidative coupling of organocuprates served in the synthesis of various biphenyls. Neither of the two metabolites seems to be responsible for the toxicity of the total *A. glaucus* extracts.

In the course of a search for food borne mycotoxins samples of mold-damaged rice were collected from a household in the village Baan Kota, Thailand, where a young boy died from an unidentified toxicosis. The microflora of these specimens consisted mainly of *Aspergillus flavus*, *A. glaucus*, *A. niger*, and an unidentified *Penicillium*. To identify the toxic agent(s), these fungi were grown on a natural medium and harvested after 2 weeks' growth, and chloroform extracts of the culture media were bioassayed with Fischer strain rats. The Penicillium extract was found to be nontoxic but extracts of both *A. flavus* and *A. glaucus* exhibited high toxicity. Not unexpectedly aflatoxin B_1 turned out to be the toxic agent in the *A. flavus* isolate. Chromatography of the *A. glaucus* concentrate over silica gel yielded two pure substances which we have named kotanin and desmethylkotanin. In this paper we report evidence leading to structures **1** and **2**, respectively, for these mold metabolites.



1, R = CH_3
2, R = H

Kotanin, obtained as colorless cubes, mp >315°, is optically active. Its ultraviolet spectrum is complex and attempts to match it with known chromophores were not immediately successful. The infrared spectrum has no absorption in the hydroxyl region but a carbonyl band at 1700 cm^{-1}. A high-resolution mass spectrum revealed a composition of $C_{24}H_{22}O_8$. Initial fears that we would be faced with a difficult and time-consuming structure problem were quickly dismissed on examination of the nuclear magnetic resonance spectrum. It revealed only eleven protons which can be assigned to an aromatic methyl group (δ 2.73), two methoxy groups (δ 3.83, 3.93), a vinylic proton (δ 5.51), and an aromatic proton (δ 6.73). These data led to the hypothesis that kotanin is a highly substituted dicoumaryl or a dichromonyl whose optical activity is caused by restricted rotation around the carbon–carbon single bond connecting the two monomer units. The high oxygen content hinted at the presence of a lactone and this was verified by base hydrolysis. A mass spectrum of the major, optically active product indicated a loss of C_4O_2, and the infrared spectrum with broad absorption at 1600 cm^{-1} agrees with the presence of a chelate. The symmetrical nature of the starting material prevails in the hydrolysis product. A six-proton singlet at δ 2.63 in the nmr spectrum is assigned to superimposed benzenoid methyl and acetyl signals. The three-proton singlet at δ 3.83 is attributed to the remaining methoxy group while one-proton resonances at δ 6.40 and 13.3 are caused by the aromatic and chelate protons, respectively. The change in elemental composition associated with the hydrolysis of kotanin can be rationalized in terms of a 4-methoxycoumarin or a 2-methoxychromone part structure.[3]

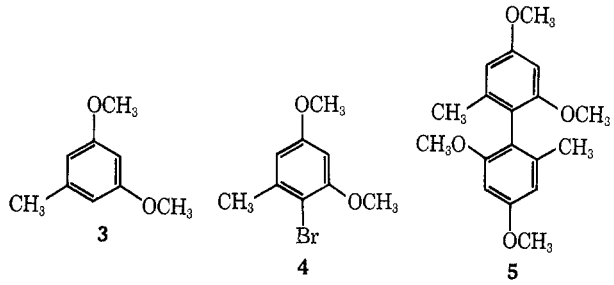


The minor product obtained on saponification of kotanin had lost the elements of C_3H_2O, and its spectral properties left no doubt that it was an intermediate on the way to the major product resulting from hydrolytic cleavage of one oxygen ring and conversion of the methoxy to a hydroxy group in the other. That two phenolic hydroxyl groups were present in the major product was confirmed by methylation to the tetramethyl ether. Structures **6**, **8**, and **15** seemed reasonable for this tetramethyl ether and to differentiate among them we turned to synthesis.

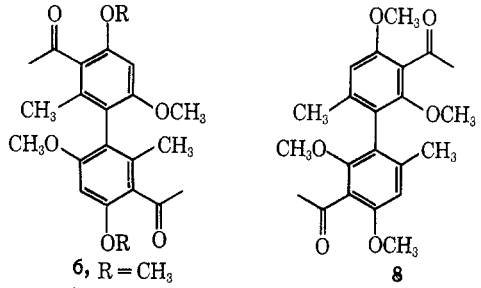
Bromination of orcinol dimethyl ether (**3**) with cupric bromide[4] rather than with bromine gave the known bromide **4**[5,6] uncontaminated by the corresponding dibromide. The bromide according to its nmr spectrum has the unsymmetrical structure. Efforts to transform it to the biphenyl **5** using Ullmann conditions failed. Metalation with butyllithium followed by

(1) Department of Chemistry.
(2) Department of Nutrition and Food Science.
(3) R. Anschütz and M. E. Scholl, *Justus Liebigs Ann. Chem.*, **379**, 333 (1911).
(4) A. W. Fort, *J. Org. Chem.*, **26**, 765 (1961).
(5) Y. Asahina and F. Fuzikawa, *Ber.*, **67**, 163 (1934).
(6) F. Fuzikawa, *ibid.*, **68B**, 72 (1935).

oxidation with cupric chloride[7,8] resulted in smooth conversion to the biphenyl **5** which was transformed further to the diketone **6** by acetylation with acetic anhydride in the presence of titanium tetrachloride. The physical properties of the racemic diketone differed from those of the optically active product obtained from kotanin by saponification and methylation.



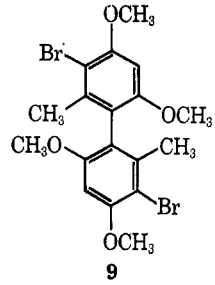
3 **4** **5**



6, R = CH_3
7, R = H

8

That the two acetyl groups had entered the benzene rings in the alternate manner leading to the diketone **8** is exceedingly unlikely, because electrophilic substitution of the biphenyl **5** with cupric bromide led to the dibromide **9** identical with the product prepared from dibromoorcinol (**10**)[6] *via* the monolithio derivative **11** and oxidation with cupric chloride.[7,8]



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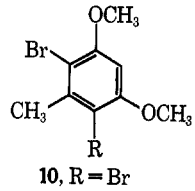
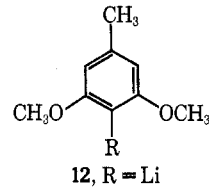
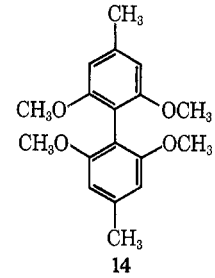


10, R = Br
11, R = Li

For the synthesis of the third diketone **15**, the tetramethoxybiphenyl **14** previously prepared in low overall yield[9] was required. A more efficient synthesis proceeding through organocopper intermediates has now been developed. Metalation of orcinol dimethyl ether[10] with butyllithium followed by oxidation of the organolithium derivative **12** with cupric chloride afforded only 18% of the desired biphenyl accompanied by 44% of the chloride **13**. Oxidation of the cuprate prepared from the lithio derivative and cuprous bromide with oxygen[7] on the other hand raised the yield of biphenyl **14** to 31%. Acetylation with acetic anhydride catalyzed by titanium tetrachloride furnished the diketone **15** identical except for optical rotation with the degradation product of kotanin. Prolonged exposure of the tetramethoxy ketone **15** to titanium tetrachloride caused selective ether cleavage to the dimethoxy ketone **16** identical as judged by spectral comparison and mixture melting point with material prepared by thermal racemization (230°, 150 min) of the optically active substance **16** derived from kotanin by base hydrolysis. With a properly substituted biphenyl in hand, we were ready to add the remaining two rings. Efforts to accomplish this in one operation using malonyl chloride[11] failed. When the carbonate **17** available from the phenol and methyl chloroformate in pyridine was subjected to the action of potassium *tert*-butoxide in *tert*-butyl alcohol, the desired cyclized product **18** was formed. Due to its highly polar nature and extreme insolubility, this intermediate could not be fully characterized and was methylated in its crude form with dimethyl sulfate in glyme in the presence of potassium carbonate. The resulting tetramethoxy compound was identical except for optical rotation with kotanin (**1**).



12, R = Li
13, R = Cl



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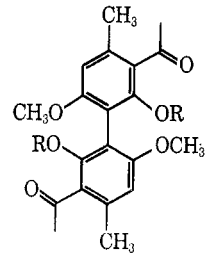
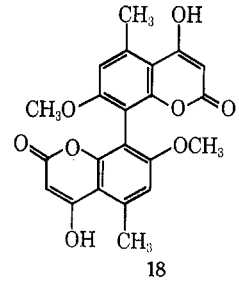


15, R = CH_3
16, R = H
17, R = $COOCH_3$



18

Methylation of monomeric β-keto lactones related to **18** with dimethyl sulfate seems to give only 4-methoxycoumarins and no 2-methoxychromones,[11,12] but structural arguments often are not convincing except in the few cases for which infrared values are reported.[13-15] Methylation with diazomethane, on the other hand, has been shown to yield both methyl ethers.[15,16] To settle this remaining question in the case of kotanin (**1**), the related 4-hydroxy-7-methoxycoumarin was methylated with diazomethane. The major product **19** with the same melting point as a compound previously assigned the coumarin structure[17] has infrared absorption at 1705 cm^{-1} and nmr absorption caused by the C_5 proton

(7) G. M. Whitesides, J. San Filippo, Jr., C. P. Casey, and E. J. Panek, *J. Amer. Chem. Soc.*, **89**, 5302 (1967).
(8) G. M. Whitesides, W. F. Fischer, Jr., J. San Filippo, Jr., R. W. Bashe, and H. O. House, *ibid.*, **91**, 4871 (1969).
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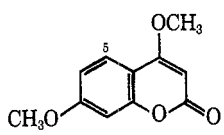
at δ 7.71. The minor product **20** now assigned the chromone structure exhibits a carbonyl band at 1635 cm^{-1} and the C_5 proton is shifted downfield by 0.3 ppm by the coplanar carbonyl group. Kotanin (**1**) has infrared absorption at 1700 cm^{-1} and its ultraviolet spectrum is essentially superimposable on that of the model coumarin **19** but very different from that of the chromone **20**. Kotanin thus has structure **1** and, to account for its optical activity and ultraviolet absorption properties, must exist in a nonplanar conformation.



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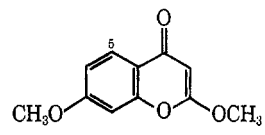

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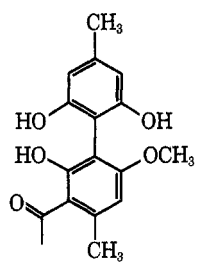

21

The second metabolite isolated from the extract of *A. glaucus* was found to possess properties similar to those of kotanin. Its mass spectrum revealed a composition of $C_{23}H_{28}O_8$ differing from kotanin by CH_2. Contrary to the latter, the ultraviolet spectrum undergoes a reversible bathochromic shift on addition of base suggesting the presence of a phenol or a 4-hydroxycoumarin. In agreement with the former postulate, methylation with diazomethane gave a single product proved to be kotanin (**1**). To substantiate this the minor metabolite was saponified. The resulting substance was not the familiar diketone **16** but a new compound whose physical properties (see Experimental Section) were in full accord with structure **21** demonstrating that the minor metabolite is indeed the phenol **2**.

The structures of kotanin and its desmethyl derivative are in good agreement with current biogenetic theory. The phenolic portion of their monomeric structures can be formed from a polyketide chain containing five acetate units with subsequent transformation involving no change in oxidation state. Oxidative phenol coupling and methylation presumably terminate the biosynthesis.

Bioassays with rats revealed pure kotanin and desmethylkotanin to be nontoxic. The agent causing the toxicity of the crude extract seems to be a trace constituent and work on its isolation is continuing.

Experimental Section

Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Ultraviolet (uv) spectra were determined on a Cary 14 recording spectrophotometer. Infrared (ir) spectra were recorded on a Perkin-Elmer Model 237 grating spectrophotometer. Nuclear magnetic resonance (nmr) spectra were measured on a Varian T-60 instrument and are given in ppm (δ) downfield from an internal tetramethylsilane standard. The abbreviations s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet, respectively. Mass spectra (ms) were determined at 70 eV on a Hitachi RMU 6E instrument; only the molecular ion is reported except when another ion is of equal or greater intensity. High resolution mass spectra were measured on a CEC 21-110B instrument. Thin layer chromatograms were made with Merck silica gel PF_{254}. Merck silica gel PF_{254}, Merck silica gel 0.05–0.02 mm, and Fischer Florisil were used for column chromatography. Vapor phase chromatographic analysis was performed on an F & M 720 instrument employing a 2-ft 10% silicon rubber column.

Isolation of Kotanin (1) and Desmethylkotanin (2).—*Aspergillus glaucus* (KOTA AG) was grown on Minute Rice and harvested after 2 weeks of growth at 30°. The culture medium was extracted in a Waring blender with chloroform, the extract was filtered, and the solvent was removed *in vacuo*. The oily residue was poured into petroleum ether (10 ml/ml of residue) and after 24 hr at 0° was filtered to afford a brown solid (980 mg). The solid was chromatographed on silica gel PF_{254} with 10% ether in methylene chloride to afford kotanin (230 mg) and desmethylkotanin (75 mg). Kotanin was recrystallized from chloroform–methanol to give white cubes: mp >315°; $[\alpha]^{25}D$ +33.1° (*c* 1.75, $CHCl_3$); uv max (EtOH) 235 nm (ε 23,400), 250 (12,180), 290 (25,100), 307 (28,800), and 316 (24,300); ir ($CHCl_3$) 3000, 2930, 2860, 1700, 1615, 1590, and 960 cm^{-1}; nmr ($CDCl_3$) δ 2.70 (3 H, s), 3.80 (3 H, s), 3.93 (3 H, s), 5.51 (1 H, s), and 6.73 (1 H, s); high resolution mass spectrum for $C_{24}H_{22}O_8$, 438.13147 (calcd), 438.13247 (found).

Desmethylkotanin was recrystallized from chloroform–hexane to give white plates: mp >315°; $[\alpha]^{25}D$ −13.3° (*c* 1.2, $CHCl_3$); uv max (EtOH) 230 nm (ε 22,300), 294 (22,300), 308 (28,300), and 315 (24,200); uv max (NaOH) 230 nm (ε 27,400), 306 (18,100), 318 (18,600), and 350 (20,400); ir ($CHCl_3$) 3000, 2950, 1700, 1618, 1590, and 1460 cm^{-1}; nmr ($CDCl_3$) 2.35 (3 H, s), 2.68 (3 H, s), 3.77 (3 H, s), 3.90 (6 H, s), 5.48 (1 H, s), 5.53 (1 H, s), 6.63 (1 H, s), 6.67 (1 H, s) and 7.55 (1 H, s); high resolution mass spectrum for $C_{23}H_{20}O_8$, 424.11581 (calcd), 424.11409 (found).

Hydrolysis of Kotanin.—A suspension of 25 mg of kotanin in 5 ml of 10% potassium hydroxide and 5 ml of dioxane was heated at reflux for 2 hr, cooled to 0°, and acidified with dilute hydrochloric acid. The resulting solution was extracted with chloroform (three 10-ml portions). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*. The yellow, oily residue was chromatographed on Florisil (1 g) using 20% ether in methylene chloride as eluent. The fast moving material **16** (15 mg, 74%) was recrystallized from ethyl acetate–hexane to give pale yellow needles: mp 220–222°, resolidified mp 272–274°; $[\alpha]^{25}D$ +109.7° (*c* 1.0, $CHCl_3$); uv max (EtOH) 235 nm (ε 21,000), 285 (23,800), and 321 (sh) (8000); uv max (NaOH) 253 nm (ε 25,200), and 310 (sh) (7560); ir ($CHCl_3$) 3000, 2860, 1600, and 1580 cm^{-1}; nmr ($CDCl_3$) δ 2.63 (6 H, s), 3.80 (3 H, s), 6.40 (1 H, s), and 13.3 (1 H, broad); mass spectrum *m/e* (rel intensity) 358 (55) and 343 (100).

The slower moving material (4 mg, 18%) was not purified further: uv max (EtOH) 249, 295, and 305 nm; ir (CH_3CN) 3620, 3540, 3000, 1705, 1595, 1260, 1120, and 830 cm^{-1}; mass spectrum *m/e* (rel intensity) 384 (82) and 369 (100).

(−)-2,2′,6,6′-Tetramethoxy-3,3′-diacetyl-4,4′-dimethylbiphenyl (15).—To a solution of hydrolysis product **16** (12 mg) and dimethyl sulfate (10 mg) in 1 ml of dry 1,2-dimethoxyethane was added finely crushed anhydrous potassium carbonate (10 mg), and the resulting suspension was heated at reflux for 3 hr. The solvent was removed *in vacuo* and to the residue was added 3 ml of water and 3ml of chloroform. The organic layer was separated and the aqueous phase was extracted with chloroform (three 3-ml portions). The combined organic extracts were dried (K_2CO_3), the solvent was removed *in vacuo*, and the residue was recrystallized from ethyl acetate–hexane to yield 8 mg (62%) of pale yellow needles: mp 136–137°; $[\alpha]^{25}D$ −3.50° (*c* 0.38, $CHCl_3$); uv max (EtOH) 232 nm (ε 18,500) and 262 (9200); ir ($CHCl_3$) 1600 cm^{-1}; nmr ($CDCl_3$) δ 2.32 (3 H, s), 2.44 (3 H, s), 3.40 (3 H, s), 3.79 (3 H, s), and 6.53 (1 H, s); mass spectrum *m/e* (rel intensity) 386 (44) and 371 (100).

Orcinol Dimethyl Ether (3).—A mixture of orcinol (6.2 g, 0.05 mol), dimethyl sulfate (13.8 g, 0.11 mol), and potassium carbonate (14 g, 0.1 mol) in 50 ml of dry 1,2-dimethoxyethane was heated at reflux under a nitrogen atmosphere for 5 hr. The reaction mixture was filtered, the solid was washed with chloroform, and the combined filtrates were washed with dilute sodium hydroxide and then with water. The solvent was removed

in vacuo and the residue was distilled to yield 6.8 g (87%) of colorless liquid, bp 70–72° (0.08 mm) [lit.[18] 240° (720 mm)].

2-Bromo-3,5-dimethoxytoluene (4).—Cupric bromide (3.3 g, 0.015 mol) was slowly added over a period of 1 hr to a vigorously stirring rolution of orcinol dimethyl ether (1.52 g, 0.01 mol) in 20 ml of dry 1,2-dimethoxyethane. The resulting green solution was stirred for an additional 1 hr and filtered to remove the precipitated salt. The solvent was removed *in vacuo* and the residue was filtered through a column of Florisil (15 g) with methylene chloride. A crystalline solid was obtained which, on recrystallization from ethanol, gave 2.0 g (87%) of colorless needles: mp 52–54° (lit.[5] 57°); uv max (EtOH) 226 nm (ε 9050) and 285 (2280); nmr ($CDCl_3$) δ 2.48 (3 H, s), 3.70 (3 H, s), 3.83 (3 H, s), and 6.35 (2 H, AB, $J < 2$ Hz).

2,2′,4,4′-Tetramethoxy-6,6′-dimethylbiphenyl (5).—To a solution of 4 (3.0 g, 0.013 mol) in 50 ml of anhydrous ether, at −78° and under a nitrogen atmosphere, was slowly added a butyllithium solution (10 ml of a 1.5 *M* solution, 0.015 mol). The reaction mixture was stirred for 1 hr and was added to a stirred suspension of anhydrous cupric chloride (2.5 g, 0.015 mol) in 5 ml of dry tetrahydrofuran at −78°. This mixture was stirred for 1 hr, warmed to room temperature, and stirred for an additional 2 hr. The mixture was then poured into dilute hydrochloric acid (10 ml); the organic phase was separated, washed with dilute hydrochloric acid (two 50-ml portions), and dried (K_2CO_3). After the solvent was removed *in vacuo*, the brown residue was chromatographed on silica gel PF_{254} (65 g) in methylene chloride to yield 450 mg (25%) of a solid. An analytical sample was recrystallized from ethanol: mp 106–108°; uv max (EtOH) 225 nm (ε 12,000) and 289 (3300); nmr ($CDCl_3$) δ 1.92 (3 H, s), 3.68 (3 H, s), 3.80 (3 H, s), and 6.43 (2 H, m).

Anal. Calcd for $C_{18}H_{22}O_4$: C, 71.50; H, 7.33. Found: C, 71.54; H, 7.35.

2,2′-Dimethyl-3,3′-diacetyl-4,4′,6,6′-tetramethoxybiphenyl (6).—To a solution of biphenyl 5 (40 mg, 0.13 mmol) in 50 ml of methylene chloride was added acetic anhydride (40 mg, 0.4 mmol) and titanium tetrachloride (200 mg, 1.1 mmol). The resulting red reaction mixture was stirred for 2 hr under nitrogen and then was poured into 50 ml of water. The organic layer was separated, the aqueous layer was extracted with methylene chloride (three 20-ml portions), and the combined extracts were dried (K_2CO_3). The solvent was removed *in vacuo* to give a brown solid which was filtered through a column of Florisil (1 g) with chloroform to yield 38 mg (75%) of product. An analytical sample was recrystallized from ethanol to give colorless needles: mp 185–187°; uv max (EtOH) 235 nm (ε 11,600), 268 (9870), and 284 (sh) (7790); ir ($CHCl_3$) 1680 cm^{-1}; nmr ($CDCl_3$) δ 1.82 (3 H, s), 2.48 (3 H, s), 3.70 (3 H, s), 3.80 (3 H, s), and 6.40 (1 H, s).

Anal. Calcd for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78. Found: C, 68.00; H, 6.90.

2,2′-Dimethyl-3,3′-dibromo-4,4′,6,6′-tetramethoxybiphenyl (9). **A.**—To a solution of biphenyl 5 (30 mg, 0.1 mmol) in 5 ml of dry 1,2-dimethoxyethane was added cupric bromide (70 mg, 0.3 mmol), and the reaction mixture was stirred for 3 hr. The solvent was removed *in vacuo* and the residue was chromatographed on a column of silica gel (1 g, 0.05–0.10 mm) packed in methylene chloride to give a crystalline material (34 mg, 75%). An analytical sample was prepared by recrystallization from ethanol: mp 230–232°; uv max (EtOH) 283 nm (ε 9600); nmr ($CDCl_3$) δ 2.1 (3 H, s), 3.7 (3 H, s), 3.95 (3 H, s), and 6.48 (1 H, s).

Anal. Calcd for $C_{18}H_{20}O_4Br_2$: C, 46.98; H, 4.38. Found: C, 46.81; H, 4.51.

B.—A solution of 2,6-dibromo-3,5-dimethoxytoluene (10)[5] (310 mg, 1 mmol) in 5 ml of anhydrous ether and 5 ml of dry tetrahydrofuran was cooled to −78° and butyllithium (0.75 ml of a 1.5 *M* solution, 1.3 mmol) was slowly added. Stirring was continued for 2 hr and then the reaction mixture was added, with vigorous stirring, to a suspension of anhydrous cupric chloride (459 mg, 3 mmol) in 3 ml of dry tetrahydrofuran. The reaction mixture was stirred for 1 hr at −78°, warmed to room temperature, and poured into 30 ml of water. The organic layer was separated, the aqueous layer was extracted with chloroform, and the combined extracts were washed with dilute hydrochloric acid and dried (K_2CO_3). Evaporation of the solvent *in vacuo* gave a pale yellow solid which was washed with 3 ml of cold (0°) ether. The residue was recrystallized from ethanol to yield 33 mg (14%) of colorless cubes, mp 229–231°, mp 228–231° when mixed with a sample prepared by method A.

2,2′,6,6′-Tetramethoxy-4,4′-dimethylbiphenyl (14). **A.**—Butyllithium solution (20 ml of a 1.5 *M* solution, 0.03 mol) was added to a solution of orcinol dimethyl ether (4.0 g, 0.026 mol) in 35 ml of dry ether under a nitrogen atmosphere. The reaction mixture was stirred for 8 hr, cooled to −78°, and then added, with vigorous stirring, to a suspension of anhydrous cupric chloride (4.6 g, 0.03 mol) in 20 ml of dry tetrahydrofuran. Stirring was continued for an additional 2 hr and the mixture was then poured into dilute hydrochloric acid. Conventional work-up in chloroform yielded an oily residue (4 g) which was chromatographed on 200 g of silica gel (0.05–0.02 mm) using methylene chloride as eluent. The first material eluted was orcinol dimethyl ether (0.3 g, 7.5%), followed by 4-chloro-3,5-dimethoxytoluene (13) (1.9 g, 44%) and finally by the desired biphenyl 14 (0.7 g, 18%). An analytical sample of 14 was prepared by recrystallization from ethanol to afford colorless needles: mp 146–148° (lit.[9] 145–146°); uv max (EtOH) 238 nm (ε 9800) and 268 (4040); nmr ($CDCl_3$) δ 2.4 (3 H, s), 3.65 (6 H, s), and 6.4 (2 H, s).

Anal. Calcd for $C_{18}H_{22}O_4$: C, 71.50; H, 7.33. Found: C, 71.39; H, 7.52.

An analytical sample of 13 was recrystallized from ethanol: mp 74–75°; uv max (EtOH) 227 nm (ε 8500) and 274 (932); nmr ($CDCl_3$) δ 2.30 (3 H, s), 3.82 (6 H, s), and 6.37 (1 H, s).

Anal. Calcd for $C_9H_{11}O_2Cl$: C, 57.91; H, 5.95. Found: C, 57.87; H, 5.96.

B.—Butyllithium (8 ml of a 1.5 *M* solution, 0.12 mol) was slowly added to a solution of orcinol dimethyl ether (1.3 g, 0.09 mol) in 30 ml of dry ether. The reaction mixture was stirred for 8 hr at room temperature, cooled to −78°, and added to a cold (−78°) suspension of flame dried cuprous bromide (1.8 g, 0.13 mol) in 15 ml of dry tetrahydrofuran. This mixture was stirred for 1 hr at −78°, oxygen was then bubbled through the solution for 1 hr, and stirring was continued for an additional 1 hr. After warming to room temperature, the reaction mixture was poured into dilute hydrochloric acid and the organic layer was separated. The aqueous layer was extracted with chloroform, the combined extracts were dried (K_2CO_3), and the solvent was removed *in vacuo*. The residue was chromatographed on 100 g of silica gel (0.05–0.20 mm) packed in methylene chloride to afford 414 mg of biphenyl 14 (31%). After recrystallization from ethanol a mixture melting point taken with the biphenyl prepared by method A showed no depression.

2,2′,6,6′-Tetramethoxy-3,3′-diacetyl-4,4′-dimethylbiphenyl (15).—A mixture of biphenyl 14 (10 mg, 0.03 mmol), acetic anhydride (10 mg, 0.1 mmol), and titanium tetrachloride (36 mg, 0.2 mmol) in 30 ml of methylene chloride was stirred for 30 min and poured into 10 ml of water. The organic layer was separated and the aqueous layer was extracted with chloroform. The combined extracts were washed with dilute sodium bicarbonate solution and dried ($MgSO_4$), and the solvent was removed *in vacuo*. Crystallization from ethanol yielded 11 mg (87%) of colorless needles: mp 162–164°; uv max (EtOH) 232 nm (ε 17,900) and 262 (9800); ir ($CHCl_3$) 1690 and 1600 cm^{-1}; nmr ($CDCl_3$) δ 2.32 (3 H, s), 2.44 (3 H, s), 3.40 (3 H, s), 3.79 (3 H, s), and 6.53 (1 H, s).

Anal. Calcd for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78. Found: C, 68.51; H, 6.70.

2,2′-Dihydroxy-3,3′-diacetyl-4,4′-dimethyl-6,6′-dimethoxybiphenyl (16).—A solution of 200 mg (0.67 mmol) of biphenyl 14, 200 mg (2 mmol) of acetic anhydride, and 800 mg (4.4 mmol) titanium tetrachloride in 50 ml of methylene chloride was stirred at room temperature for 24 hr and heated at reflux for an additional 4 hr. The red reaction mixture (containing a precipitate formed during the reaction) was poured into water, the aqueous mixture was extracted with chloroform, and the combined organic extracts were reextracted with dilute sodium hydroxide solution. The basic layer was washed with chloroform and acidified with dilute hydrochloric acid. A resulting precipitate was extracted with chloroform, the combined organic extracts were dried (Na_2SO_4), and the solvent was removed *in vacuo* to afford 225 mg (95%) of product, mp 272–275°. The uv and ir of this product were identical with those of material prepared from kotanin. Because of low solubility of the racemate in chloroform, trifluoroacetic acid was used as solvent for the nmr spectrum (TFA) δ 2.76 (3 H, s), 2.86 (3 H, s), 3.94 (3 H, s), and 6.73 (1 H, s).

2,2′-Dihydroxy-3,3′-diacetyl-4,4′-dimethyl-6,6′-dimethoxybiphenyl Bismethyl Carbonate (17).—To a solution of bisaceto-

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phenone 16 (100 mg, 0.28 mmol) in 10 ml of pyridine was slowly added methyl chloroformate (250 mg, 2.6 mmol). The reaction mixture was heated at 75° for 4 hr and poured into dilute hydrochloric acid, and the aqueous phase was extracted with chloroform. The combined organic extracts were washed with dilute hydrochloric acid and dried ($NaSO_4$), and the solvent was removed *in vacuo*. Crystallization of the residue from benzene–hexane yielded colorless needles (124 mg, 94%): mp 152–154°; uv max (EtOH) 238 nm (ε 25,800) and 258 (14,900); ir ($CHCl_3$) 1780, 1700, and 1615 cm^{-1}; nmr ($CDCl_3$) δ 2.40 (3 H, s), 2.44 (3 H, s), 3.64 (3 H, s), 3.74 (3 H, s), and 6.67 (1 H, s).

Anal. Calcd for $C_{24}H_{26}O_{10}$: C, 60.76; H, 5.52. Found: C, 60.57; H, 5.51.

Kotanin (1).—To 10 ml of dry *tert*-BuOH was added 100 mg of potassium. The mixture was stirred at room temperature for 2 hr and heated at reflux for 2 hr (until all the potassium had reacted). The mixture was cooled and 118 mg (0.25 mmol) of carbonate 17 was added, and the resulting mixture was heated at reflux for 2 hr, cooled and poured into water, and extracted with chloroform. The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo* to yield 86 mg (82%) of a pale yellow solid. A mixture of 41 mg of the above coumarin, 70 mg of dimethyl sulfate, and 100 mg potassium carbonate in 10 ml of glyme was heated at reflux for 4 hr, cooled, and evaporated to dryness *in vacuo*. To the residue was added 10 ml of water and this was extracted with chloroform. The combined extracts were dried (K_2CO_3) and evaporated *in vacuo* to afford a yellow solid which was chromatographed by preparative tlc to yield 13 mg (29%) of kotanin. Recrystallization from chloroform–methanol gave pale yellow cubes, mp >315°. The uv, ir, and nmr spectra were identical with those of natural kotanin.

Racemization of the Bisacetophenone 16.—Bisacetophenone 16 (8 mg, $[\alpha]^{25}D$ +109.7°) was heated under nitrogen at 200° for 30 min. The recovered product was still optically active, $[\alpha]^{25}D$ +51.0° (*c* 0.4, $CHCl_3$) and consequently was heated for a further 2 hr. After this time it had $[\alpha]^{25}D$ 0° (*c* 0.4, $CHCl_3$), mp 272–275°; mixture melting point with synthetic bisacetophenone 16 not depressed. The ir spectrum and tlc behavior were identical with those of synthetic material.

4-Hydroxy-7-methoxycoumarin.—To 1.24 g (10 mmol) of *m*-methoxyphenol in 60 ml of methylene chloride was added 1.40 g (10 mmol) of malonyl dichloride followed by careful addition of 1 ml of titanium tetrachloride. The reaction mixture was stirred at room temperature for 24 hr and poured into ice–water and the organic phase was separated. The aqueous layer was extracted with chloroform and the combined organic extracts were washed with water and extracted with dilute sodium hydroxide. The aqueous extracts were washed with chloroform and acidified with dilute HCl to give a voluminous precipitate which was filtered off to yield 1.0 g (60%) of the coumarin, mp 246–253° dec. Sublimation at 120° (0.05 mm) gave white needles: mp 249–253° dec (lit.[17] 256°); uv max (EtOH) 238 nm (ε 10,400), 246 (9200), 280 (10,900), and 298 (16,700); ir ($CHCl_3$) 3600, 3400, 1700, and 1600 cm^{-1}; nmr (DMSO-d_6) δ 3.87 (3 H, s), 5.52 (1 H, s), 7.00 (2 H, m), and 7.80 (1 H, m).

4,7-Dimethoxycoumarin (19) and 2,7-Dimethoxychromone (20).—To 680 mg (3.54 mmol) of 4-hydroxy-7-methoxycoumarin in 50 ml of MeOH was added an excess (20 mmol) of freshly distilled diazomethane in ether. The reaction mixture was stirred at room temperature for 12 hr and the solvent was removed *in vacuo*. The residue (690 mg) was chromatographed on 65 g of silica gel PF_{254} in chloroform to yield 270 mg of coumarin 19 and 87 mg of chromone 20 (total yield of 50%).

A sample of the coumarin was sublimed (100°, 0.05 mm) to afford colorless needles: mp 157–159° (lit.[17] 156°); uv max (EtOH) 215 nm (ε 21,600), 275 (8590), 302 (15,800), and 310 (12,700); ir ($CHCl_3$) 1705 and 1620 cm^{-1}; nmr ($CDCl_3$) δ 3.86 (3 H, s), 3.97 (3 H, s), 5.56 (1 H, s), 6.86 (2 H, m), and 7.71 (1 H, d, J = 8 Hz).

An analytical sample of the chromone was prepared by sublimation (100°, 0.05 mm) and recrystallization from ethanol to give plates: mp 156–158°, mmp (with coumarin) 120–148°; uv max (EtOH) 217 nm (ε 7650), 236 (5610), 242 (5510), and 272 (14,800); ir ($CHCl_3$) 1635 and 1610 cm^{-1}; nmr ($CDCl_3$) δ 3.78 (3 H, s), 3.85 (3 H, s), 5.43 (1 H, s), 6.85 (2 H, m), and 8.0 (1 H, d, J = 8 Hz).

Anal. Calcd for $C_{11}H_{10}O_4$: C, 64.07; H, 4.89. Found: C, 63.90; H, 4.85.

Methylation of Desmethylkotanin (2) to Kotanin (1).—To 3 mg of desmethylkotanin in 0.5 ml of methanol was added a large excess of freshly prepared diazomethane in ether. The reaction mixture was stirred at room temperature for 10 min followed by removal of the solvent *in vacuo* to yield 3 mg of material identical with natural kotanin as judged by tlc behavior and ir and uv spectra.

Hydrolysis of Desmethylkotanin (2) to the Ketone 21.—A suspension of 9 mg of desmethylkotanin in 3 ml of 10% potassium hydroxide and 2 ml of dioxane was heated at reflux for 7 hr under a nitrogen atmosphere. The reaction mixture was cooled, acidified, and extracted with chloroform. The combined extracts were dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield 6 mg of crude product which was chromatographed on 1 g of silica gel (2% methanol–chloroform) to yield 4 mg (65%) of phenol 21. An analytical sample was recrystallized from ethyl acetate–hexane: mp 206–208°; uv max (EtOH) 215 nm (ε 25,600), and 277 (10,500); uv max (NaOH) 251 nm (ε 20,000) and 319 (4800); ir ($CHCl_3$) 3600, 3400, 1600, and 1560 cm^{-1}; nmr ($CDCl_3$) δ 2.35 (3 H, s), 2.72 (6 H, s), 3.90 (3 H, s), 4.92 (2 H, broad), 6.58 (3 H, s), and 13.7 (1 H, broad); mass spectrum *m/e* (rel intensity) 302 (65) and 287 (100); high resolution mass spectrum for $C_{17}H_{18}O_5$, 302.11542 (calcd), 302.11502 (found).

Registry No.—**1**, 27909-08-6; **1** racemate, 27909-09-7; **2**, 27909-10-6; **4**, 13321-73-8; **5**, 20261-64-7; **6**, 27921-27-3; **9**, 27921-28-4; **13**, 27971-69-3; **14**, 27921-29-5; **15**, 27909-11-1; **15** racemate, 27909-12-2; **16**, 27909-13-3; **16** racemate, 27909-14-4; **17**, 27921-30-8; **19**, 17575-27-8; **20**, 27921-32-0; **21**, 27921-33-1; 4-hydroxy-7-methoxycoumarin, 17575-15-4.

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