ANTIMICROBIAL NEOLIGNANS OF *SASSAFRAS RANDAIENSE* ROOTS

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ABSTRACT.—Two antimicrobial neolignans, magnolol (**1**) and its previously unreported isomer, isomagnolol (**2**), were isolated from the roots of *Sassafras randaiense*. The structure of **2** was established by its spectra and partial synthesis.

As a part of a research program dealing with screening local and foreign flora for biologically active compounds, an alcoholic extract of the roots of *Sassafras randaiense* (Hayata) Rehder (Lauraceae) was found to exhibit antimicrobial activity against a number of microorganisms. These included *Staphylococcus aureus, Mycobacterium smegmatis, Saccharomycetes cerevisiae,* and *Trichophyton mentagrophytes.* This paper describes the isolation and chemical characterization of the two compounds responsible for this activity: magnolol (**1**) and a novel compound that has been named isomagnolol (**2**).

Ethanolic extraction of the powdered whole roots of *S. randaiense* collected in Taiwan provided an extract that exhibited antimicrobial activity. Partitioning between water and chloroform concentrated the activity in the organic phase. Silica gel chromatography of the latter provided two active fractions: fraction 2 and fraction 4 (see Experimental section).

Fraction 4 was examined first, in view of its higher yield and because it was easier to purify by crystallization from ether-*n*-hexane to give prisms. mp, 101-102°C. Examination of its physical and spectral properties (see Experimental section) suggested that it was the neolignan magnolol (**1**). Its identity was confirmed by direct comparison with and authentic sample of **1** previously isolated in our laboratories (1).

Fraction 2 was obtained as a light yellow oil that was essentially homogeneous on tlc. It was further purified by rechromatography on silica gel to provide the analytical sample of molecular formula $C_{18}H_{18}O_2$. Its ir spectrum ($CHCl_3$) exhibited a hydroxyl absorption band at 3520 cm^{-1}, while the pmr spectrum ($CDCl_3$) upon comparison with that of magnolol (**1**), suggested the presence of two nonequivalent allyl groups. The cmr spectrum ($CDCl_3$) (table 1) exhibited three oxygenated aromatic carbon signals at δ 155.4, 145.9, and 143.6. The relatively high field positions of the last pair suggested a catechol moiety of the type found in structure **2** or alternative structure **3**.[1] Assignments of the remaining carbon signals would be consistent with either structure.

To distinguish between structures **2** and **3**, the compound was acetylated to give **5** as a colorless oil. The cmr of **5** (table 1) showed a distinct downfield shift of the singlet, due to C-4 relative to the phenolic compound. This is consistent only with structure **2** (2). Furthermore, dehydroxylation of **2** by formation of the tetrazole **6**, followed by catalytic hydrogenation yielded the ether **7**, whose pmr clearly ruled out the symmetrical structure that would be expected from **3**.

Methylation of **2** by treatment with dimethylsulfate in alkaline medium provided **4** as a colorless oil whose cmr spectrum (table 1) was consistent with the proposed struc-

[1]The fact that this compound possessed either structure **2** or **3** was, at least, partially confirmed by subjecting its methyl ether (**4**) to Birch reduction (3). The product was analyzed by gc-ms to give two main products. One of them showed a retention time and ms identical to those of chavicol (**11**). Unfortunately, the other product was not identical with estragole (**8**), and its mass was two units higher. Apparently it was an overreduction product of **8**.

TABLE 1. Cmr assignments of isomagnolol (**2**) and its derivatives **4** and **5**.

Carbon	2	4	5
1	145.9s[e]	150.0s[f]	140.4s[g]
2	143.6s[e]	145.3s[f]	148.6s[g]
3	119.2d	121.5d	120.7d
4	132.6s	133.8s[a]	139.2s
5	124.8d	124.6d	124.0d
6	116.2d[h]	113.2d[h]	123.6d[g,h]
1′	155.4s[i]	156.6s[i]	155.7s[i]
2′	117.9d	117.1d	118.3d
3′	129.9d	129.6d	129.8d
4′	135.3s	133.2s[a]	135.0s
5′	129.9d	129.6d	129.8d
6′	117.9d	117.1d	118.3d
α	39.4t	39.3t[b]	39.7t[a]
β	137.5d	137.7d[c]	137.5d[b]
γ	115.8t[a]	115.7t[d]	116.2t[c]
α′	39.4t	39.5t[b]	39.5t[a]
β′	137.5d	137.4d[c]	136.8d[b]
γ′	115.6t[a]	115.5t[d]	115.7t[c]
$-\overset{O}{\overset{\|}{C}}CH_3$			168.7s
$-C\overset{O}{\overset{\|}{C}}H_3$			20.4[j]
$-OCH_3$		56.3q	

[a,b,c,d]Interchangeable within the same column.

[e]Assigned by inspection of the proton coupled spectra in which the signal at δ 145.9 showed a triplet (J^3 = 7.0 Hz) indicating three bond coupling to H-3 and H-5. The signal at δ 143.6 was masked by overlap with the signal at 137.5.

[f]A long-range selective decoupling (lspd) experiment (irradiation at δ 3.78) confirmed this assignment; the δ 150.0 signal sharpened significantly while the signal at δ 145.3 remained unchanged.

[g]Assigned on the basis of the documented upfield shift of the ipso carbon and downfield shift of the ortho carbons upon acetylation (2).

[h]Inspection of the proton coupled spectrum allowed assignment of these carbons since in each compound the carbon appears as a doublet (δ 116.2 J^1 = 161 Hz, δ 113.2 J^1 = 162 Hz, and δ 123.6 J^1 = 159 Hz) with no three bond coupling to any hydrogens.

[i]These signals appear as triplets in their respective proton coupled spectra each being three bond coupled to its respective H-3′ and H-5′ with the following coupling constants, δ 155.4 J^3 = 7.0 Hz, 156.6 J^3 = 8.0 Hz, and 155.7 J^3 = 7.0 Hz.

[j]This signal appears as a quartet (J = 7.0 Hz) in the proton coupled spectrum evidently due to two bond coupling with the acetate methyl.

ture. The formation of **4** also served another purpose, as the next atttempt to confirm unambiguously the structure of **2** focused on the total synthesis of **4**. This was accomplished by brominating[2] estragole (**8**) followed by debromination by treatment with zinc in acetic acid to yield **9**.[3] The pmr of **9** (see Experimental section) unambiguously established its structure. The Ulmann reaction of **9** with chavicol (**11**) in the presence of cupric oxide (5) provided a product in 27% yield[4] indistinguishable from **4**, thus confirming the structure of **2**.

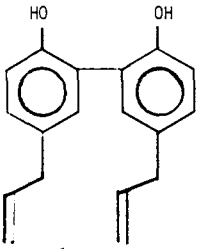
[2]Originally, it was planned to produce **9** by methylating 3-bromo-4-methoxy-phenylacetaldehyde using CH_2I_2/Mg (4). However, attempts to synthesize this aldehyde from the analogous alcohol invariably led to overoxidation to 3-bromo-*p*-anisaldehyde.

[3]The novel, rearranged dibromide (**10**) was obtained as a byproduct of this reaction (see Experimental section).

[4]Attempts to improve the yield by the use of pentafluorophenyl copper (5) were unsuccessful.

Compounds of the type of **1** and **2** are seldom found together. The literature cites only one recent example about the occurrence of the corresponding dehydroeugenols in the trunk wood of *Ocotea cymbarum* (6).

The antimicrobial activity of **2** is slightly inferior to that of **1**, which was previously reported (7). Its minimum inhibitory concentrations (MIC) against *S. aureus*, *M. smegmatis*, *S. cerevisiae*, and *T. mentagrophytes* were 3.12 μg/ml, 1.56 μg/ml, 25 μg/ml, and 1.56 μg/ml, respectively.



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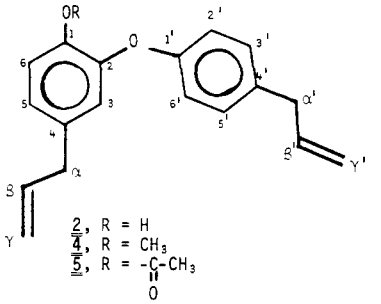

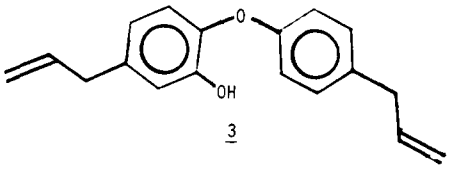

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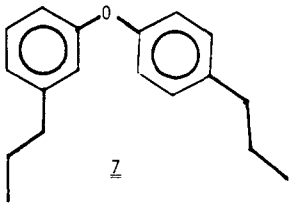

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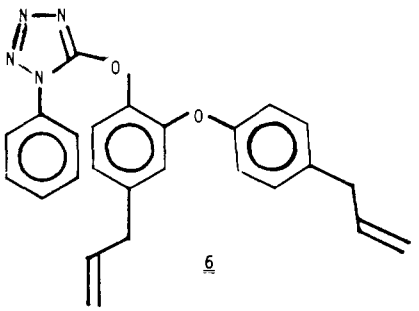

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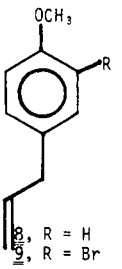

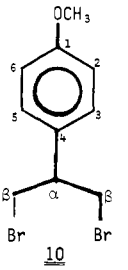

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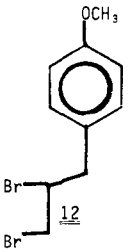

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EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were taken in capillaries on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. The ir spectra were determined on a Beckman IR-33 recording infrared spectrophotometer; pmr spectra were recorded on Varian Model EM390 nuclear magnetic resonance spectrometer at 90 MHz operating at 35°C with $CDCl_3$ as the solvent and tetramethyl-

silane as an internal standard with chemical shifts reported as δ values; cmr (15.03 MHz) spectra were recorded on JEOL FX-60 instrument with a 45° pulse angle, repetition rates between 5-10s, and 8 K data points, and using $CDCl_3$ as solvent. Proton noise decoupled spectra were obtained by broad-band (1 KHz) irradiation. Single frequency off-resonance decouplings were conducted by centering the decoupling frequency 100 Hz downfield from the signal for tetramethylsilane. The long-range selective proton decoupling (lspd) was carried out by centering the decoupler at the corresponding proton resonance and recording the spectra at very low power levels. Mass spectra were taken on a Finnigan 3200 mass spectrometer. Gc-ms were obtained by the same instrument, using a column of 2% OV-17 run isothermally at 175°C. Elemental analyses were done by Galbraith Laboratories, Inc., Knoxville TN 37921. Spot detection on tlc plates was achieved by spraying with 0.5% aqueous $KMnO_4$ or by viewing under uv light.

PLANT EXTRACTION.—Whole roots of *S. randaiense* were collected in Taiwan in February 1981 by Mr. Wen-Shyong Li of the National Pingtung Institute of Agriculture, who also identified the plant material. A voucher specimen is kept at the herbarium of the Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677. The powdered plant material (821 g) was extracted by 95% ethanol at ambient temperature. Evaporation of the alcoholic extract *in vacuo* at 40°C. left 126 g of a dark oily residue,[5] which was partitioned between 600 ml of $CHCl_3$ and four 100-ml portions of H_2O. The chloroform phase was dried (anhydrous Na_2SO_4) and evaporated to leave 44 g of an oil.[5]

ISOLATION OF MAGNOLOL (**1**) AND ISOMAGNOLOL (**2**).—The chloroform solubles (20 g) obtained above were chromatographed on 450 g of silica gel 60, using chloroform as solvent. The composition of the fractions obtained was monitored by tlc on silica gel G plates using $CHCl_3$ as solvent and fractions of similar composition were pooled together giving fraction 1 (0.089 g), fraction 2 (0.806 g), fraction 3 (0.580 g), and fraction 4 (13.700 g). The column was then washed with methanol to give 0.752 g of residue. Antimicrobial evaluation revealed that the activity resided mainly in fractions 2 and 4.

Fraction 2 which was homogeneous on tlc (one spot, Rf 0.72 on silica gel G plates using $CHCl_3$ as solvent) but slightly colored was decolorized by dissolving in $CHCl_3$ and filtration on silica gel. Evaporation of the filtrate provided 0.777 g of (**2**) obtained as colorless oil; ir, ν max ($CHCl_3$) 3520, 1500, and 1214 cm^{-1}; pmr, δ ($CDCl_3$) 3.21 (d, 2H J = 6.0 Hz). 3.36 (d, 2H J = 7.0 Hz) 5.02 (m, 4H), 5.45 (s, exchangeable), 5.91 (m, 2H), and the seven aromatic protons between 6.70-7.19; ms, M^+ at *m/z* 266 (26%) with the base peak at *m/z* 117. For the cmr see table 1.

Anal. calcd for $C_{18}H_{18}O_2$: C, 81.17; H, 6.81. Found: C, 81.21; H, 6.88.

In later runs, isomagnolol (**2**) was more conveniently isolated from the plant material as follows: *Sassafras randaiense* ground roots (200 g) were extracted with 95% ethanol. The solvent was removed *in vacuo* at 40° leaving a residue that was taken up in 500 ml $CHCl_3$. The $CHCl_3$ was filtered to remove insoluble material and then removed *in vacuo* to leave 14.5 g of a dark oil. Flash chromatography (8) on silica gel using ether-hexane (1:4) as solvent gave 10.35 g magnolol (**1**) and 0.44 g of isomagnolol (**2**).

Fraction 4 was also essentially one spot on tlc (Rf 0.41, using silica gel G plates, and $CHCl_3$ as solvent) and was recrystallized from ether-*n*-hexane to give almost colorless prisms (11.11 g) of **1**; mp, 101-102° [Lit. mp (1), 101.5-102°, with ir, ms, pmr, and cmr spectra identical with those of magnolol (**1**) (1), and their mixed melting point was not depressed.

Anal. calcd for $C_{18}H_{18}O_2$: C, 81.17; H, 6.81. Found: C, 81.03; H, 6.85.

ACETYLATION OF ISOMAGNOLOL (**2**) TO **5**.—Isomagnolol (**2**) (200 mg) was dissolved in a solution containing 1.0 ml pyridine and 1.0 ml acetic anhydride. The mixture was stirred overnight at room temperature. After the usual work-up, 217 mg of a yellow oil was obtained, which was decolorized by filtering over a small pad of silica gel 60 using 20% ether in hexane as solvent. The ir ($CHCl_3$) showed no hydroxyl absorption bands but exhibited carbonyl absorption at ν max 1758 cm^{-1}; pmr δ ($CDCl_3$) 2.10 (s, 3H), 3.28 (d, 2H J = 4.0 Hz), 3.37 (d, 2H J = 4.0 Hz), 5.04 (br d, 4H J = 12.0 Hz), 5.93 (m, 2H), and the seven aromatic protons absorbed between 6.79-7.23; for the cmr, see table 1; ms, M^+ at *m/z* 308 (0.37%) with base peak at *m/z* 117.

Anal. calcd for $C_{20}H_{20}O_3$: C, 77.90; H, 6.54. Found: C, 78.12; H, 6.67.

CONVERSION OF ISOMAGNOLOL (**2**) TO **6**.—Anhydrous potassium carbonate (500 mg) and 5-chloro-1-phenyl-1H-tetrazole (67 mg) were added to 2 ml of an acetone solution containing 89.5 mg of isomagnolol (**2**). The mixture was refluxed until tlc analysis using 20% ether-hexane as solvent showed it to be complete (10 h). Filtration, followed by removal of the acetone and flash chromatography using 20%

[5]The antimicrobial activity of these fractions was tested against the following microorganisms: *Bacillus subtilis* (ATCC 6633), *Saphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442), *Mycobacterium smegmatis* (ATCC 607), *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763). *Aspergillus niger* (ATCC 16888), and *Trichophyton mentagrophytes* (ATCC 9972). Routine qualitative screening and evaluation of antimicrobial activities was accomplished as previously described (7).

ether in hexane, led to the isolation of 121 mg of pure **6** as one spot on tlc (Rf 0.21); ν max ($CHCl_3$) 1591 cm^{-1} and 1495 cm^{-1}; pmr δ ($CDCl_3$) 3.33 (d, 4H J = 6.0 Hz), 5.07 (br d, 4H, J = 10.0 Hz), 5.91 (m, 2H), and 12 aromatic protons between δ 6.76-7.77; cmr δ ($CDCl_3$), 159.7 (s), 155.0 (s), 147.4 (s), 142.9 (s), 140.3 (s), 137.2 (d), 136.4 (d), 135.4 (s), 133.3 (s), 129.8 (d), 129.5 (d), 129.0 (d), 124.4 (d), 122.0 (d), 121.0 (d), 117.9 (d), 116.5 (t), 39.5 (q); ms, M^+ at m/z 410 (3%) with base peak at m/z 117.

Anal. calcd for $C_{25}H_{22}O_2N_4$: C, 73.19; H, 5.41; N, 13.66. Found: C, 73.21; H, 5.43; N, 13.77.

CATALYTIC HYDROGENATION OF **6** TO **7**.—Tetrazole (**6**) (72 mg) was dissolved in 10 ml of absolute ethanol, and 10% palladium on carbon (70 mg) was added. The mixture was hydrogenated under a pressure of 20 psi for 18 h. Filtration followed by evaporation of the solvent left an oily residue that was purified by filtration over a short bed of silica gel 60 using 20% ether in *n*-hexane to give 42 mg of pure **7**; ν max ($CHCl_3$ 2928, 1500, and 1248 cm^{-1}; pmr δ ($CDCl_3$) 0.92 (t, 6H J = 5.0 Hz), 1.59 (m, 4H), 2.57 (t, 4H J = 8.0 Hz), nonsymmetrical aromatic signals for eight protons between 6.73-7.33; ms, M^+ at m/z 254 (70%) with the base peak at 225.

Anal. calcd for $C_{18}H_{22}O$: C, 84.99; H, 8.72. Found: C, 84.79; H, 8.82.

METHYLATION OF ISOMAGNOLOL (**2**) TO **4**.—Isomagnolol (**2**) (101 mg) was dissolved in 5 ml of 95% ethanol, and 1.0 ml of dimethylsulfate was added. The reaction was kept basic with 4 N potassium hydroxide, and after stirring for 3 h, the solvent was removed *in vacuo* and the solution acidified. After the usual work-up, 107 mg of **4** were obtained as a colorless oil; ν max ($CHCl_3$) 1498 cm^{-1} and 1265 cm^{-1}; with no hydroxyl absorption bands; pmr δ ($CDCl_3$) 3.30 (d, 2H J = 6.0 Hz), 3.70 (d, 2H J = 6.0 Hz), 3.79 (s, 3H), 5.07 (br d, 4H J = 11.0 Hz), 5.93 (m, 2H), and seven aromatic protons between 6.83-7.27; ms, M^+ at m/z 280 (30%) with the base peak at m/z 117.

Anal. calcd for $C_{19}H_{20}O_2$: C, 81.39; H, 7.19. Found: C, 81.44; H, 7.11.

BROMINATION OF ESTRAGOLE (**8**) TO **9**.—Method I: estragole (**8**) (5.0 g) was dissolved in 50 ml CCl_4, and bromine (1.73 ml) was added to give a mixture of **10** and **12** in the ratio 40:60, respectively, as determined by gc-ms analysis (retention times 1:20, 1:09). More bromine (1.73 ml) was added to the solution, and the mixture was stirred overnight. Removal of the solvent *in vacuo*, followed by filtration of the product over silica gel 60 using 5% ether in benzene as solvent, gave 13 g of a mixture of tribrominated material as determined by gc-ms analysis (retention times 4:50 and 5:26 for the two tribrominated components).[6] After dissolving 5 g of the tribrominated mixture in 40 ml of ether, acetic acid (0.83 ml) followed by zinc dust (1.3 g) were added, and the reaction mixture was refluxed for 4 h and then stirred for 18 h at room temperature. The mixture was filtered, and the filtrate was extracted with 50 ml of water and ether and evaporated to give 2.5 g of a clear oil, which, by tlc analysis (40% benzene in hexane), consisted of two major components (uv). Visualization of the plate with $KMnO_4$ spray reagent revealed only one reactive component (Rf 0.59), which was isolated by chromatography over silica gel G using 40% benzene in hexane as the solvent system and giving 1.5 g of pure **9**. The ir spectrum showed absorption bands at ν max ($CHCl_3$) 1598 cm^{-1}, 1490 cm^{-1}, 1275 cm^{-1}, and 1252 cm^{-1}; pmr δ ($CDCl_3$) 3.31 (d, 2H J = 7.0 Hz), 3.86 (s, 3H), 5.10 (br d, 2H J = 12.0 Hz), 5.93 (m, 1H), 6.82 (d, 1H J = 8.0 Hz), 7.03 (dd, 1H J = 8.0, 2.0 Hz), 7.39 (d, 1H J = 2.0 Hz) cmr δ ($CDCl_3$), 154.4 (s), 137.0 (d), 133.8 (s), 133.4 (d), 128.5 (d), 116.0 (t), 112.1 (d), 111.7 (s), ms, M^+ at m/z 226 (46%) M+2 at m/z 228 (46%) with the base peak at m/z 147.

Method II: estragole (**8**) (5.0 g) was dissolved in 100 ml acetic acid and to this solution, 12.96 g of pyridinium hydrobromide perbromide (purity 80+%-Aldrich) was added and the mixture stirred until the crystals of the pyridinium hydrobromide perbromide had dissolved (approximately 30 minutes). This procedure produced a mixture of **10** and **12** in the ratio 20:80 as determined by gc ms (see above) analysis. More pyridinium hydrobromide perbromide (12.96 g) was added to the acetic acid soluton and the mixture stirred overnight. Most of the acetic acid was removed *in vacuo* leaving a red oil that was taken up in benzene (100 ml), washed once with 50 ml of water and three times with 50 ml of saturated aqueous sodium bicarbonate solution, and finally dried over anhydrous sodium sulfate. Removal of the benzene *in vacuo* left 10.47 g of a pale yellow oil. Treatment of this oil in 80 ml ether with 2.1 g zinc dust and 1.7 ml of acetic acid under reflux for 4 h followed by stirring at room temperature for 18 h gave 6.44 g of a residue. Chromatography of the residue as in Method I led to the isolation of 2.10 g of **9**, which was identical to the material obtained from Method I.

ISOLATION AND CHARACTERIZATION OF **10**.—The mixture of the dibromides **10** and **12** obtained from Method I was found to be inseparable by column chromatography, and **10** could only be obtained in pure form by converting **12** back to estragole (**8**) as follows: The mixture (2.0 g) was dissolved in 20 ml of

[6]Retention times of the components of the tribrominated mixture were determined isothermally at 200°C.

ether containing 0.40 ml of acetic acid. Zinc dust (0.44 g) was then added, and the mixture was stirred for 14 h at room temperature. Work-up as above gave 1.23 g of a mixture of estragole (**8**) and **10**, which were separated by column chromatography over silica gel using 32% benzene in *n*-hexane as the solvent system. This procedure yielded 340 mg of estragole (**8**) and 740 mg of **10**. The ir of **10** showed absorption bands at ν max ($CHCl_3$) 1605 cm^{-1}, 1508 cm^{-1}, and 1250 cm^{-1}; pmr δ ($CDCl_3$) 3.27 (m, 1H), 3.67 (d, masked by signal at 3.72, 4H). 3.72 (s, 3H), 6.84 (d, 2H = 9.0 Hz), 7.12 (d, 2H J = 9.0 Hz): cmr δ ($CDCl_3$) 159.2 (s, C-1), 131.6 (s, C-4), 128.5 (d, C-3 and 5), 114.2 (d, C-2 and 6). 55.2 (q, OCH_3), 48.1 (d, C-α), 35.8 (t, C-β); ms, M^+ at *m/z* 306 (6%), M+2 at *m/z* 308 (12%), M+4 at *m/z* 310 (6%), with base peak at *m/z* 134 (9).

Anal. calcd for $C_{10}H_{12}OBr_2$: C, 38.99; H, 3.93, br, 51.89. Found: C, 40.12; H, 4.11; Br, 52.00.

COUPLING OF **9** WITH CHAVICOL (**11**).—In 1.5 ml of freshly distilled collidine, 60 mg of **9**, 59 mg of **11**, 36 mg of potassium carbonate, and 21 mg of CuO were refluxed under N_2 for 13 h. The mixture was poured into 50 ml of 10% hydrochloric acid and extracted twice with 50 ml of benzene. The combined benzene layers were washed with 50 ml of saturated aqueous sodium bicarbonate, 50 ml water, dried over anhydrous sodium sulfate and evaporated, leaving 113 mg of a dark oily residue. This residue was chromatographed over a silica gel G column using 40% benzene in *n*-hexane as the solvent system giving 15.7 mg of synthetic **4** which was indistinguishable from the material obtained previously and described above by the methylation of **2**. Also 12.7 mg of the starting material **9** was isolated.

BIRCH REDUCTION OF THE ETHER **4**.—Sodium metal (80 mg) was stirred in liquid ammonia (15 ml) under N_2 and cooled in dry ice-acetone bath. The ether **4** (49 mg) was dissolved in 1.0 ml of dry tetrahydrofuran and added to the reaction mixture, which was then stirred for 2 h. Next, the reaction mixture was worked up by the addition of absolute ethanol (0.7 ml), by acidification with dilute HCl, and then by ether extraction. Evaporation of the dry (anhydrous Na_2SO_4) ether extract left 43 mg of an oily residue, which was subjected to gc ms analysis (see general experimental procedures) to give two bands with retention times 5.22 and 7.04. The mass spectrum of the latter band showed M^+ at *m/z* 134 (base peak) and was identical to that of chavicol (**11**). The former band however showed M^+ at *m/z* 150 and was not further characterized.

ACKNOWLEDGMENTS

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