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O-Methylation Effect on the Carbon-13 Nuclear Magnetic Resonance Signals of *ortho*-Disubstituted Phenols and Its Application to Structure Determination of New Phthalides from *Aspergillus silvaticus*

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The *O*-methylation effect on the carbon-13 nuclear magnetic resonance chemical shifts of *ortho*-disubstituted phenols was investigated. In phenols with nonconjugated *ortho*-disubstituents (**1**—**10**), *O*-methylation caused a downfield shift by an average of 5.2 ppm for the *ortho*-carbons (C-2 and -6), and a downfield shift by an average of 4.6 ppm for the *para*-carbon (C-4). These shift values are significantly different from those observed in *ortho*-monosubstituted and *ortho*-nonsubstituted phenols. This regularity is very useful for the spectral interpretation of some natural products with an *ortho*-disubstituted phenol group and for the determination of the position of the methoxy group in such compounds.

Two new phthalides, silvaticol (**15**) and *O*-methylsilvaticol (**16**), were isolated along with nidulol (**17**) from *Aspergillus silvaticus*. In the course of the structure determination of these compounds, the *O*-methylation effects of *ortho*-mono- and *ortho*-disubstituted phenols were successfully applied to these phthalides. The structures of silvaticol and *O*-methylsilvaticol were determined as 6-hydroxy-4-methoxy-5-methylphthalide and 4,6-dimethoxy-5-methylphthalide, respectively.

Keywords——^{13}C-NMR; *O*-methylation shift; *ortho*-disubstituted phenol (NMR); *Aspergillus silvaticus*; phthalide; silvaticol; nidulol; 6-hydroxy-4-methoxy-5-methylphthalide; 4,6-dimethoxy-5-methylphthalide

In the course of screening *Aspergillus* species for antimicrobial metabolites, two new phthalides, named silvaticol (**15**) and *O*-methylsilvaticol (**16**), were isolated along with nidulol (**17**)[1] from the culture filtrate of *Aspergillus silvaticus* FENNELL and RAPER IFO 8173. Chemical investigation of silvaticol (**15**) led us to alternative structures with different locations of the *O*-methyl group. In order to determine the position of the methoxy group we investigated the *O*-methylation shifts of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) signals of *ortho*-disubstituted phenols (**1**—**11**). The results were quite different from the reported *O*-methylation effect in *ortho*-monosubstituted phenols.[2] This report deals with the effect of *O*-methylation on the ^{13}C-NMR signals of *ortho*-disubstituted phenols in general, and its application to the structural investigation of new phthalides from *Aspergillus silvaticus*.

Effect of *O*-Methylation of *ortho*-Disubstituted Phenols

In the phenols (**1**—**13**) studied in this paper, the carbon bearing the hydroxyl group which suffers methylation is always numbered C-1, and the carbon linked to the *ortho*-substituents, C-2 and -6 (see Chart 1). The carbon linked to the formyl group in **11** and **13** is numbered C-2. Compound numbers suffixed with **m** indicate the methyl ether of the corresponding phenols; **5m** is 2,3-dimethoxyphenol.

The assignment of chemical shifts of the carbon nuclei was carried out grossly on the basis of the signal multiplicity in off-resonance decoupled spectra and the signal amplitude in

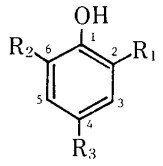


Chart 1

1: $R_1=R_2=CH_3$, $R_3=H$
2: $R_1=R_2=R_3=CH_3$
3: $R_1=R_2=Cl$, $R_3=H$
4: $R_1=R_2=R_3=Cl$
5: $R_1=OH$, $R_2=OCH_3$, $R_3=H$
6: $R_1=R_2=OCH_3$, $R_3=H$
7: $R_1=R_2=OCH_3$, $R_3=CHO$
8: $R_1=R_2=OCH_3$, $R_3=COCH_3$
9: $R_1=R_2=OCH_3$, $R_3=CH=CH-COOH$ (*trans*)
10: $R_1=R_2=OCH_3$, $R_3=CH=CH-COOCH_3$ (*trans*)
11: $R_1=CHO$, $R_2=OCH_3$, $R_3=H$
12: $R_1=R_2=OH$, $R_3=H$
13: $R_1=CHO$, $R_2=OH$, $R_3=H$

proton-coupled spectra without nuclear Overhauser effect (NOE). The following carbon-proton long-range couplings in the proton-coupled spectrum were used to assist in the assignments of the aromatic carbons; among the coupling constants (J) between carbons and protons in the aromatic ring system, $^3J_{C-H}$ is usually larger than $^2J_{C-H}$ and $^4J_{C-H}$.[3a] The coupling constant ($^3J_{C-H}$) between aromatic carbon and methoxy protons and the coupling constant ($^2J_{C-H}$) between aromatic carbon and formyl proton are also large.[3b] Thus, the assignments of the following carbon pairs were confirmed by examination of the proton-coupled spectra: C-1 and C-2 (C-6) in **1**, **1m**, **3**, **3m**, **6**, **6m** and **12**; C-4 and C-3 (C-5) in **1**, **1m**, **3**, **3m**, **6**, **6m** and **12**; C-1 and C-4 in **2**, **2m**, **4**, **4m**, **8** and **8m**; C-1, C-2 (C-6) and C-4 in **7**, **7m**, **9**, **9m**, **10** and **10m**; C-4 and C-3 or C-5 in **5**, **5m**, **11**, **11m** and **13**; C-1, C-2 and C-6 in **5**, **5m**, **11** and **13**; C-2, and C-1 or C-6 in **11m**. The assignments of C-3 (C-5) and the vinylic carbons in **9**, **9m**, **10** and **10m** were confirmed by examination of proton selective decoupling spectra. The assignments of the other aromatic carbons in **5**, **5m** and **11** were made by application of *O*-demethylation or *O*-methylation shift values of *ortho*-monosubstituted phenols[2a] to **6m**, **12** and **13**,[4] and these assignments were also supported by comparison of the proton-coupled spectra of similar compounds (**6**, **6m** and **12**). The chemical shifts of C-1 and C-6 in **11m** were determined by comparison of the proton-coupled spectra of **11m** and the trideuteriomethyl (CD_3) ether of **11**. The observed chemical shifts of the aromatic carbons of eleven *ortho*-disubstituted phenols (**1**—**11**) and their methyl ethers (**1m**—**11m**) are listed in Table I. The *O*-methylation shift values (Δ) in Table I were calculated by subtracting the carbon chemical shifts of phenols from the corresponding chemical shifts of thier methyl ethers.

From the observed chemical shifts of the aromatic carbons in the *ortho*-disubstituted phenols **1**—**10** and their methyl ethers **1m**—**10m**, methylation of the phenolic hydroxyl group gave rise to downfield shifts (from +0.4 to +3.4 ppm; mean, +1.8 ppm) for the *ipso*-carbons (C-1), downfield shifts (from +4.5 to +6.1 ppm; mean, +5.2 ppm) for the *ortho*-carbons (C-2 and -6), and also downfield shifts (from +3.5 to +5.5 ppm; mean, +4.6 ppm) for the *para*-carbons (C-4) in DMSO-d_6. The *meta*-carbons (C-3 and -5) are not greatly affected by *O*-methylation: the shift values range from −0.6 to +0.4 ppm (mean, −0.2 ppm). The *O*-methylation shift values (Δ) for the two *ortho*-carbons (C-2 and -6) and the *para*-carbon (C-4) in *ortho*-disubstituted phenols were about +5 ppm, whereas in *ortho*-monosubstituted phenols the shift values (Δ) for the substituted *ortho*-carbon (C-2), the *ortho*-methine carbon (C-6) and the *para*-carbon (C-4) were +1 ppm, −4 ppm and +1 ppm, respectively.[2a] The *O*-methylation shift values (Δ) for the *ipso*-carbons (C-1) range from +0.4 to +3.4 ppm (mean, +2.0 ppm) and are comparable to those in the case of *ortho*-monosubstituted phenols,[2b] though the phenols (**7**, **8**, **9** and **10**) with *para*-conjugation systems gave rise to a rather smaller shift by +0.4 to +1.4 ppm on *O*-methylation. The above regularity is very useful for the spectral interpretation of an *ortho*-substituted phenol and its methyl ether and for the determination of the position of a methoxy group in *ortho*-substituted phenols.

In only one example of a phenol possessing a formyl group at C-2 (**11**), did the *O*-methylation shift value (Δ) for C-3 (−2.9 ppm) deviate significantly in amplitude from those obtained for **1**—**10**. The carbon-3 of compound **11** is located *ortho* to C-2 carrying the

TABLE I. Chemical Shifts of Aryl Carbons and *O*-Methylation Shifts (in DMSO-d_6)

Compound and shift value (Δ)	Carbon No.						OMe	Others
	C-1	C-2	C-3	C-4	C-5	C-6		
1	153.2	124.2	128.1	119.1	128.1	124.2		16.6 (2,6-Me)
1m	156.6	130.0	128.4	123.3	128.4	130.0	58.8	15.5 (2,6-Me)
Δ	+3.4	+5.8	+0.3	+4.2	+0.3	+5.8		
2	151.6	124.9	129.6	128.6	129.6	124.9		16.8 (2,6-Me), 20.3 (4-Me)
2m	154.4	129.5	129.0	132.1	129.0	129.5	58.8	15.4 (2,6-Me), 20.1 (4-Me)
Δ	+2.8	+4.6	−0.6	+3.5	−0.6	+4.6		
3	149.1	122.3	128.4	120.7	128.4	122.3		
3m	151.5	128.4	128.8	125.4	128.8	128.4	60.1	
Δ	+2.4	+6.1	+0.4	+4.7	+0.4	+6.1		
4	148.7	123.2	128.0	123.4	128.0	123.2		
4m	150.7	128.8	128.0	128.2	128.0	128.8	59.6	
Δ	+2.0	+5.6	0	+4.8	0	+5.6		
5	134.9	146.5	110.1	119.7	104.5	149.3	56.5	
5m	137.0	151.2	109.8	123.9	103.9	153.8	55.8, 60.2	
Δ	+2.1	+4.7	−0.3	+4.2	−0.6	+4.5		
6	135.7	148.2	105.7	118.1	105.7	148.2	55.9	
6m	137.7	153.2	105.6	123.6	105.6	153.2	55.8, 59.9	
Δ	+2.0	+5.0	−0.1	+5.5	−0.1	+5.0		
7	142.5	148.4	107.2	127.7	107.2	148.4	56.2	191.2 (C=O)
7m	142.9	153.2	106.6	131.6	106.6	153.2	55.8, 60.0	191.3 (C=O)
	+0.4	+4.8	−0.6	+3.9	−0.6	+4.8		
8	141.1	147.5	106.2	127.6	106.2	147.5	56.1	26.1 (COMe), 196.1 (C=O)
8m	141.9	152.6	105.7	132.2	105.7	152.6	55.9, 60.0	26.3 (COMe), 196.4 (C=O)
Δ	+0.8	+5.1	−0.5	+4.6	−0.5	+5.1		
9	138.4	148.4	106.3	125.0	106.3	148.4	56.4	116.4 (C-8),[a] 145.1 (C-7),[a] 168.3 (C=O)
9m	139.8	153.4	106.0	130.2	106.0	153.4	56.1, 60.4	118.7 (C-8), 144.5 (C-7), 168.2 (C=O)
Δ	+1.4	+5.0	−0.3	+5.2	−0.3	+5.0		
10	138.7	148.2	106.3	124.7	106.3	148.2	56.2	114.8 (C-8), 145.5 (C-7), 167.3 (C=O), 51.3 (ester)
10m	139.7	153.2	105.9	129.7	105.9	153.2	56.0, 60.1	117.0 (C-8), 144.8 (C-7), 166.9 (C=O), 51.3 (ester)
Δ	+1.0	+5.0	−0.4	+5.0	−0.4	+5.0		
11	151.0	122.4	121.3	119.3	117.7	148.4	56.0	193.2 (C=O)
11m	152.8	129.3	118.4[b]	123.9	118.3[b]	152.1	55.7, 61.5	189.4 (C=O)
Δ	+1.8	+6.9	−2.9	+4.6	+0.6	+3.7		

m: methyl ethers of the corresponding phenols.
a) C-7 and C-8 in **9**, **9m**, **10** and **10m** are β and α to the carbonyl group respectively.
b) Assignments may be reversed.

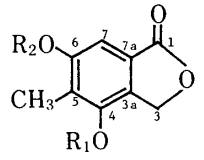
conjugated substituent.

The effect on C-2, C-4 and C-6 caused by the methylation of *ortho*-disubstituted phenols has been employed without generalization by some researchers in interpretation of the spectra of particular examples of phenolic natural products.[5] This paper is the first to point out the wide applicability of the effect. It is noteworthy that Dhami and Stothers,[6] and Buchanan[7] reported that C-2, -4 and -6 of some *ortho*-disubstituted anisoles resonate at lower fields than those calculated on the basis of the additivity rule of substituent effects. They also suggested that this phenomenon might arise from the steric effect of the methoxy carbon on C-2 and -6.

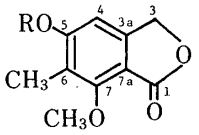
Structures of Silvaticol (15) and *O*-Methylsilvaticol (16)

Silvaticol (**15**), mp 213 °C, $C_{10}H_{10}O_4$, was isolated as the main component from *Aspergillus silvaticus*. Compound **15** was very similar to nidulol (**17**) in all of the spectroscopic data except ^{1}H-NMR signals at δ 3.91 (3H, s), and 7.04 (1H, s), so **15** should be the isomer of **17**. An aromatic proton at δ 7.04 in **15** appeared at 0.5 ppm downfield from the corresponding proton in **17**, which suggested that this aromatic proton was located at the *peri*-position with respect to a carbonyl group in **15**. Upfield shift of the methoxy protons from δ 4.08 in **17** to δ 3.91 in **15** also indicates that the methoxy group was not located at the *peri*-position to a carbonyl group in **15**. On methylation, **15** afforded a monomethyl ether (**16**) which was identical with *O*-methylsilvaticol, mp 154 °C, $C_{11}H_{12}O_4$, also obtained from the above fungus. Furthermore, on permanganate oxidation, **16** gave a phthalic anhydride (**19**) which was identical with 4,6-dimethoxy-5-methylphthalic anhydride derived from **17** by methylation and permanganate oxidation.[1] From the above results, the structure of *O*-methylsilvaticol was confirmed to be as shown in **16**, and therefore that of silvaticol was determined to be as shown in **15**, excluding the position of the *O*-methyl group.

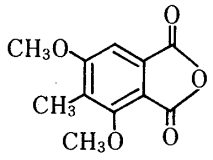
In order to determine the position of the methoxy group in silvaticol (**15**), we applied the *O*-methylation shifts of the *ortho*-monosubstituted phenols[2] and the *ortho*-disubstituted



14 : $R_1=R_2=H$
15 : $R_1=CH_3$, $R_2=H$
16 : $R_1=R_2=CH_3$



17 : $R=H$
18 : $R=CH_3$



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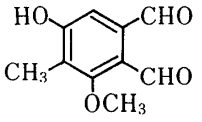

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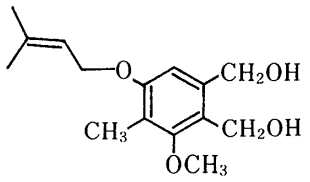

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Chart 2

TABLE II. Carbon Chemical Shifts of Some Phthalides (in DMSO-d_6)

Example	Compound and shift value (Δ)	Carbon No. C-3a	C-4	C-5	C-6	C-7	C-7a	OCH_3	Others
1	**17**	148.2	103.2	162.6	117.4	157.4	107.2	61.3	8.3 (6-CH_3), 69.3 (C-3), 168.1 (C-1)
	18	149.1	99.9	163.6	118.8	156.6	108.9	61.6, 56.4	8.5 (6-CH_3), 68.8 (C-3), 168.1 (C-1)
	Δ	+0.9	−3.3	+1.0	+1.6	−0.8	+1.7		
2	**15**	125.0	152.8	121.6	157.2	103.5	124.1	58.4	9.5 (5-CH_3), 68.5 (C-3), 170.4 (C-1)
	16	127.2	152.4	122.9	159.0	99.8	124.4	58.5, 56.1	9.5 (5-CH_3), 68.5 (C-3), 170.3 (C-1)
	Δ	+2.2	−0.4	+1.3	+1.8	−3.7	−0.3		
3	**14**	123.1	150.0	118.1	157.3	100.9	124.2		9.2 (5-CH_3), 68.0 (C-3), 170.8 (C-1)
	15	125.0	152.8	121.6	157.2	103.5	124.1	58.4	
	Δ	+1.9	+2.8	+3.5	−0.1	+2.6	−0.1		

phenols mentioned in the early part of this paper to the above phthalides (**14**—**18**). The assignments of the carbon chemical shifts in **14**—**18** were carried out on the basis of the signal multiplicity in off-resonance decoupled spectra and the additivity rule of substituent effects[3a] in phthalides reported by Hughes *et al.*[8] First, we measured the ^{13}C-NMR spectra of the known phthalide **17** and its methyl ether (**18**). The *O*-methylation shift values (Δ) on corresponding carbons from C-3a to C-7a were calculated and are listed in Table II (example 1). The above shift values were consistent with those of simple *ortho*-monosubstituted phenols.[2] In particular, *O*-methylation caused an upfield shift by 3.3 ppm for the protonated carbon atom (C-4), which should be *ortho* to the methylated hydroxyl group in **17**. Thus, the *O*-methylation effect of *ortho*-substituted phenols can also be applied to naturally occurring phthalides. Then, we measured the ^{13}C-NMR spectra of **14**, **15** and **16**, and the calculated results (Δ) are listed in Table II (examples 2 and 3). The protonated aromatic carbon atom (C-7) was observed at 3.7 ppm upfield in example 2 and at 2.6 ppm downfield in example 3 after methylation. These results show that the methylated positions were located *ortho* (C-6) to C-7 in **15** and *para* (C-4) to C-7 in **14**, considering the *O*-methylation effects of *ortho*-mono- and *ortho*-disubstituted phenols. The shift values (Δ) for the other aromatic carbons in examples 2 and 3 were also comparable with those of the *ortho*-mono- and *ortho*-disubstituted phenols, which supported the above determination of the positions of the methylated hydroxyl groups in **14** and **15**. From the above results, the structure of silvaticol was determined as the 6-demethyl ether of **16**, *i.e.*, 6-hydroxy-4-methoxy-5-methylphthalide (**15** in Chart 2).

Silvaticol (**15**) was obtained by the chemical conversion of quadrilineatin (**20**) from *Aspergillus quadrilineatus*[9] and zinniol (**21**) from *Alternaria Zinniae*.[10] It is interesting that **15** and **16** were isolated along with **17** from the same fungus, in relation to the biogenesis of these phthalides. Silvaticol (**15**) slightly inhibited the growth of *Helminthosporium maydis* at the concentration of 25 μg/ml.

Experimental

Melting points are uncorrected. Infrared (IR) and ultraviolet (UV) spectra were taken with a Hitachi 215 spectrophotometer and a Hitachi 124 spectrophotometer, respectively. Mass spectra (MS) were obtained on a JEOL JMS-D 300 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a JEOL JNM-FX 100 spectrometer operating at 99.6 MHz for proton and at 25.05 MHz for carbon-13 spectra (data points, 8 k; spectral width, 5000 Hz; flip angle, 30°; 3 s between pulses). The ^{13}C-NMR spectra were recorded in dimethyl sulfoxide (DMSO)-d_6 at room temperature using tetramethylsilane as an internal standard. Proton-coupled spectra of the symmetrical phenols and anisoles were obtained by using an electronic gating system without the nuclear Overhauser effect. Proton-coupled spectra of the compounds possessing the phenolic hydroxyl group were recorded both in DMSO-d_6 and in a mixture of DMSO-d_6 and D_2O (1 : 1). The chemical shifts are given on the δ scale (ppm).

Materials——**2m**, **10** and **10m** were prepared from commercially available compounds by the procedures described below. The purity and structure of the products were confirmed by thin-layer chromatography (Merck, Kiesel gel 60 F_{254} precoated plates), by GC-MS (JEOL JMS-D 300; 2% OV-1 on Chromosorb W (60—80 mesh); column temp. 100 °C) and by ^{1}H-NMR (DMSO-d_6 as the solvent). Other materials used were commercial products.[11]

Synthesis of 2m——Dimethyl sulfate (120 mM) was added to **2** (100 mM) dissolved in 10 ml of 15% NaOH. The mixture was stirred at room temp. for 5 h, and poured into ice-water. After Et_2O extraction of the product, the Et_2O layers were washed with 10% NaOH and then H_2O, and dried over $CaCl_2$. After removal of the solvent, the product **2m** was obtained. MS *m/z*: 150 (M^+). ^{1}H-NMR δ: 2.17 (9H, s), 3.60 (3H, s), 6.77 (2H, br s).

Synthesis of 10 and 10m——Conc. H_2SO_4 (0.4 ml) was added to the acid (**9** or **9m**) (1.5 g) dissolved in 15 ml of abs. MeOH. The mixture was refluxed for 5 h then allowed to stand overnight. The precipitate formed was recrystallized to give the corresponding ester (**10** or **10m**). **10**: Colorless needles from EtOH, mp 91—92 °C. MS *m/z*: 238 (M^+). ^{1}H-NMR δ: 3.71 (3H, s), 3.81 (6H, s), 6.50 (1H, d, J=15.9 Hz), 7.00 (2H, s), 7.56 (1H, d, J=15.9 Hz). **10m**: colorless plates from aq. MeOH, mp 92—92.5 °C. MS *m/z*: 252 (M^+). ^{1}H-NMR δ: 3.71 (3H, s), 3.73 (3H, s), 3.82 (6H, s), 6.60 (1H, d, J=16.0 Hz), 7.01 (2H, s), 7.58 (1H, d, J=16.0 Hz).

Synthesis of the Trideuteriomethyl Ether of 11——Dimethyl sulfate-d_6 (35 mM) was added to **11** (30 mM) dissolved in 4 ml of 10% NaOH. The mixture was refluxed for 4 h and poured into ice-water. After Et_2O extraction of the product, the Et_2O layers were washed with 10% NaOH and then H_2O, and dried over $CaCl_2$. After removal of the

solvent, the product was obtained by distillation at 90 °C/2 mmHg. Colorless crystals, mp 48—50 °C. MS *m/z*: 169 (M^+). ^{1}H-NMR δ: 3.88 (3H, s), 7.05—7.37 (3H, m), 10.31 (1H, s).

Isolation of Silvaticol (15) and *O*-Methylsilvaticol (16)——*Aspergillus silvaticus* FENNEL and RAPER (IFO 8173) was cultivated at 27 °C for 15 d in Czapek-Dox medium. The culture filtrate (50 l) was extracted with CH_2Cl_2 at pH 2 and at pH 9. The acidic extracts were chromatographed on silica gel with benzene–acetone (100 : 1) to obtain nidulol (**17**) (85 mg), and with benzene–acetone (50 : 1) to obtain silvaticol (**15**) (719 mg) and *O*-methylsilvaticol (**16**) (16 mg). From the mycelia, **15** (10 mg) and mannitol (63 mg) were also isolated. **15**: Colorless needles, mp 213 °C from MeOH. IR ν_{max}^{KBr} cm^{-1}: 3325 (OH), 1730 (C=O). UV λ_{max}^{EtOH} nm (log ε): 215 (4.56), 256 (3.81), 303 (3.49). MS *m/z*: 194 (M^+), 165. ^{1}H-NMR δ in $CDCl_3$: 2.23 (3H, s, 5-Me), 3.91 (3H, s, 4-OMe), 5.39 (2H, s, 3-H), 7.04 (1H, s, 7-H). *Anal.* Calcd for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 62.24; H, 5.18. **16**: Colorless needles, mp 154 °C from benzene or MeOH. IR ν_{max}^{KBr} cm^{-1}: 1740 (C=O). MS *m/z*: 208 (M^+), 179. ^{1}H-NMR δ in $CDCl_3$: 2.20 (3H, s, 5-Me), 3.89 (6H, s, OMe), 5.38 (2H, s, 3-H), 7.08 (1H, s, 7-H). *Anal.* Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.39; H, 5.81. **17**: Colorless needles, mp 208 °C from $CHCl_3$. IR ν_{max}^{KBr} cm^{-1}: 3325 (OH), 1735 (C=O). MS *m/z*: 194 (M^+). ^{1}H-NMR δ in $CDCl_3$: 2.19 (3H, s, 6-Me), 4.08 (3H, s, 7-OMe), 5.15 (2H, s, 3-H), 6.59 (1H, s, 4-H).

Methylation of 15——**15** (65 mg) was methylated with CH_3I (20 ml) and Ag_2O (500 mg) at room temp. for 5 h. After removal of Ag_2O, the evaporated residue was crystallized from MeOH to give a monomethyl ether (**16**) (43 mg), which was proved to be identical with *O*-methylsilvaticol by IR, TLC, and ^{1}H-NMR comparisons and mixed fusion.

Demethylation of 15——**15** (20 mg) was demethylated with BBr_3 (1 ml) in CH_2Cl_2 (4 ml) for 5 min under reflux. The mixture was poured into ice-water and extracted with AcOEt. Crystallization of the evaporated residue gave *O*-demethylsilvaticol (**14**) (14 mg). MS *m/z*: 180 (M^+), 151. ^{1}H-NMR δ in DMSO-d_6: 2.07 (3H, s, 5-Me), 5.14 (2H, s, 3-H), 6.71 (1H, s, 7-H), 9.73 (1H, br s, OH).

Methylation of 17——**17** (40 mg) was methylated with CH_3I (18 ml) and Ag_2O (285 mg) at room temp. for 20 h. After removal of Ag_2O, the evaporated residue was chromatographed on silica gel to give **18** (14 mg). Colorless needles, mp 152 °C. IR ν_{max}^{KBr} cm^{-1}: 1740 (C=O). MS *m/z*: 208 (M^+). ^{1}H-NMR δ in $CDCl_3$: 2.14 (3H, s, 6-Me), 3.91 (3H, s, 5-OMe), 4.04 (3H, s, 7-OMe), 5.18 (2H, s, 3-H), 6.62 (1H, s, 4-H).

Permanganate Oxidation of 16——**16** (36 mg) was refluxed in 0.1 N NaOH (10 ml) for 1 h and then oxidized with $KMnO_4$ (942 mg) dissolved in 2 ml of H_2O at room temp. for 2 h. The reaction mixture was acidified and extracted with $CHCl_3$. Then the evaporated residue was refluxed with Ac_2O (0.5 ml) for 30 min. The final products were chromatographed on silica gel to give a phthalic anhydride (**19**) (3.2 mg). Colorless crystals, mp 162 °C. IR ν_{max}^{KBr} cm^{-1}: 1845, 1770 (COOCO). MS *m/z*: 222 (M^+). ^{1}H-NMR δ in $CDCl_3$: 2.21 (3H, s, 5-Me), 3.99 (3H, s, 6-OMe), 4.17 (3H, s, 4-OMe), 7.17 (1H, s, 7-H). The above phthalic anhydride was identical with the product synthesized from **18** by permanganate oxidation in the manner described above, on the basis of IR, TLC and ^{1}H-NMR comparisons.

References and Notes

1) P. J. Aucamp and C. W. Holzapfel, *J. South Afr. Chem. Inst.*, **21**, 26 (1968).
2) *a*) Average shift values of *O*-methylation in reference 2*b*: +2.0 ppm for *ipso*-carbon, +1.1 ppm for substituted *ortho*-carbon and *para*-carbon, and −4.1 ppm for *ortho*-methine carbon. The *meta*-carbons are not greatly affected by *O*-methylation; *b*) M. Fujita, M. Nagai, and T. Inoue, *Chem. Pharm. Bull.*, **30**, 1151 (1982).
3) *a*) G. C. Levy, R. L. Lichter, and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance Spectroscopy," 2nd ed., John Wiley and Sons, Inc., New York, 1980, pp. 102—135; *b*) J. L. Marshall, "Carbon–Carbon and Carbon–Proton NMR Couplings: Applications to Organic Stereochemistry and Conformational Analysis," Verlag Chemie International, Florida, 1983, pp. 42—51.
4) Carbon chemical shifts (δ_c, ppm) of **12** were assigned as follows. C-1, 133.7; C-2 and -6, 146.8; C-3 and -5, 108.2; C-4, 119.7. Application of the *O*-methylation effect gives calculated chemical shifts of 109.3, 119.7, and 104.1 ppm for C-3, -4 and -5 of **5**, respectively. If **6m** is demethylated to yield **5m**, chemical shifts of the aromatic carbons of **5m** can be calculated as follows. C-1, 136.6; C-2, 151.2; C-3, 109.7; C-4, 123.6; C-5, 104.5; C-6, 153.2. Inspection of the proton-coupled spectrum of **13** disclosed that signals at δ_c 119.8, 122.6, 146.8 and 149.4 ppm are attributable to C-4, -2, -6 and -1, respectively, and that those at δ_c 121.0 and 122.0 ppm are attributable to C-3 and -5. Taking into consideration the *O*-methylation effect and the off-resonance decoupled spectrum of **11**, the carbon chemical shifts of **11** were best assigned as listed in Table I.
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9) J. H. Birkinshaw, P. Chaplen, and R. Lahoz-Oliver, *Biochem. J.*, **67**, 155 (1957).
10) A. N. Starratt, *Can. J. Chem.*, **46**, 767 (1968).
11) **1m**, **3m**, **5**, **5m**, **8** and **9** were purchased from Aldrich Chemical Co., **9m** was from Nakarai Chemicals Ltd., **12** was from Wako Pure Chemical Industries Ltd., and the others were from Tokyo Kasei Co., Ltd.