Chloro- and Bromophenols from Cultures of *Mycena alcalina*

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Received July 12, 2006

Three new chlorinated phenols have been isolated from mycelial cultures of the mushroom *Mycena alcalina*. Their structures were determined by mass spectrometry and 1D and 2D NMR experiments. Addition of bromide to the medium resulted in the production of the corresponding brominated phenols. In addition, small amounts of the nonhalogenated precursor were also isolated, indicating that the halogenated metabolites are generated by a regioselectively operating halogenase.

Fungi belonging to the genus *Mycena* are known to produce a variety of secondary metabolites, such as the pyrroloquinoline alkaloid haematopodin,[1] a number of polyines[2] and terpenoids,[3] and several of the antifungal strobilurins and oudemansins.[4] However, no secondary metabolites have been reported from the species *Mycena stipata* Maas Gesteranus & Schwöbel, formerly known under the name *M. alcalina* [Fr.] Kummer.[5] Therefore, we screened cultures of *M. alcalina*[6] for new secondary metabolites using GC-MS and have identified three chlorinated natural products from the typical isotope pattern of the molecular ions caused by the presence of chlorine. In this paper, we describe the isolation and structural elucidation of these secondary metabolites, which we have decided to name alcalinaphenols A (**1**), B (**2**), and C (**3**). In addition, the substrate specificity of the putative halogenase was investigated with respect to its brominating capability. On addition of potassium bromide to the medium, three brominated metabolites, the alcalinaphenols D (**4**), E (**5**), and F (**6**), were generated instead of the corresponding natural products **1**, **2**, and **3**, respectively.

The alcalinaphenols were extracted with MeOH from 8-week-old mycelial cultures of *M. alcalina*[6] grown on MEA medium and purified by preparative and semipreparative HPLC on RP-18.

Alcalinaphenol A (**1**) exhibits a molecular ion at 188.02387 in the HREIMS, corresponding to a molecular formula of $C_8H_9ClO_3$, indicating the presence of four double-bond equivalents. The molecular formula is confirmed by the ^{13}C NMR spectrum, which shows six carbons in the aromatic region and two carbons in the aliphatic region at δ_C 19.54 and 60.76. The ^1H NMR spectrum reveals one proton in the aromatic region, while the two singlets in the aliphatic region at δ_H 2.20 and 3.79 belong to a methyl and an *O*-methyl group, respectively. Considering these results and the molecular formula, **1** is an aromatic compound with five substituents, one chloro, one methyl, one methoxy, and two hydroxy groups. The substitution pattern of alcalinaphenol A is deduced unambiguously from the correlations in the ROESY, HMBC, and HSQC spectra (Figure 1). A correlation between the singlet at δ_H 2.20 and the singlet at δ_H 6.50 in the ROESY spectrum indicates that the aromatic CH group is located adjacent to the carbon carrying the CH_3 group. The three aromatic carbons with the highest shift values should carry the hydroxy and methoxy groups. In the HMBC there are only strong correlations from the methyl group to the other three aromatic carbons. Hence, the hydroxy groups and the methoxy group must be located *meta* and *para* to the methyl substituent, and the chloro substituent is located *ortho* to the methyl substituent. Since there is no HMBC correlation from the aromatic proton to the aromatic carbon that carries the methoxy group, and no correlation from the methoxy protons to any other proton in the ROESY either, the methoxy group must be located *ortho* to the chloro substituent and *para* to the aromatic proton.

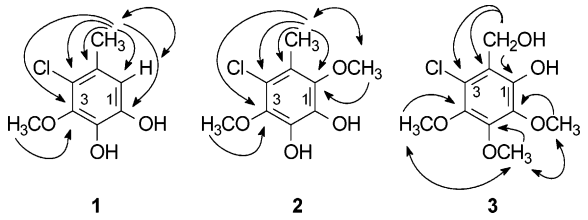

Figure 1. HMBC (→) and NOE (↔) correlations of alcalinaphenols A−C (**1**−**3**).

Alcalinaphenol B (**2**) exhibits a molecular ion at 218.03399 corresponding to a molecular formula of $C_9H_{11}ClO_4$. The EIMS shows a shift of 30 mass units in comparison to **1** in most fragments. Aromatic protons are absent from the ^1H NMR, but two singlets at δ_H 3.71 and 3.77, each representing an *O*-methyl group, are present in **2** instead of only one in **1**. Therefore, alcalinaphenol B possesses an additional methoxy group. According to the HSQC, HMBC, and ROESY spectra, this methoxy group is located adjacent to the methyl substituent and replaces the aromatic proton of alcalinaphenol A (Figure 1).

Alcalinaphenol C (**3**) possesses a molecular ion at 248.04529, corresponding to a molecular formula of $C_{10}H_{13}ClO_5$. The UV spectrum is very similar to that of **1** and **2**; therefore **3** should also be a phenol. Compared to **2**, alcalinaphenol C (**3**) possesses an additional oxygen, an additional carbon, and two additional hydrogen atoms. In the ^1H NMR spectrum, three resonances in the range between δ_H 3.5 and 4.0 indicate the presence of three methoxy groups instead of the two in **2**. The signal of the methyl substituent at δ_H 2.19 in alcalinaphenol B (**2**) is absent in **3**. Instead, a new resonance comprising two protons is visible at δ_H 4.77, indicating the presence of a CH_2OH substituent replacing the methyl substituent of **2**. The positions of the methoxy groups of **3** were elucidated from the correlations in the ROESY and the HMBC spectra (Figure 1). Only two NOE correlations from the methoxy group at δ_H 3.91 to the methoxy groups at δ_H 3.84 and 3.78 indicate that the methoxy group at δ_H 3.91 is flanked by those at δ_H 3.84 and 3.78, while there are no NOE correlations between the CH_2-OH substituent and any methoxy group. Therefore, the chloro substituent and the hydroxy group must be located adjacent to the CH_2OH group, while the three methoxy substituents must be located *meta* and *para* to the CH_2OH group. The correlations in the HSQC and HMBC spectra allow an almost complete assignment of the carbon and proton resonances and confirm the structure of **3**.

When *M. alcalina* was grown on MEA medium enriched with KBr, new phenols were detected in the GC-MS after extraction of the mycelium with MeOH and subsequent pertrimethylsilylation of the dried crude extract. Three monobrominated phenols were

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10.1021/np0603368 CCC: $33.50
Published on Web 11/20/2006

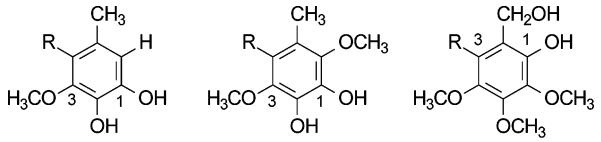

Figure 2. Alcalinaphenols A−F (**1**−**6**) and nonhalogenated phenols **7** and **8** from cultures of *M. alcalina*.

identified from the characteristic isotope pattern of bromine. In addition, traces of metabolites containing two bromine atoms were also found by GC-MS.

The major component, the pertrimethylsilylated alcalinaphenol E (**5**), exhibits a molecular weight of 406 [M (^{79}Br)]$^+$. In comparison to pertrimethylsilylated **2**, most fragment ions are shifted by 44 mass units. Hence, **5** is identical to **2**, except that chlorine has been replaced by bromine (Figure 2). This result was confirmed by isolating and purifying **5** by HPLC and recording its ^{1}H, HSQC, HMBC, and ROESY NMR spectra.

The two monobrominated components **4** and **6** were generated in minute amounts that did not permit their isolation and characterization by NMR. However, their structures could be deduced from the mass spectra of the corresponding pertrimethylsilyl derivatives. The pertrimethylsilyl derivative of alcalinaphenol D (**4**) exhibits a molecular weight of 376 [M (^{79}Br)]$^+$ and a shift of most fragment ions in comparison to the pertrimethylsilylated **1** of 44 mass units. Hence, **4** is identical with **1**, except that chlorine has been replaced by bromine (Figure 2). Compound **6**, alcalinaphenol F, is related to **3** since its pertrimethylsilyl derivative exhibits a molecular ion at 436 [M (^{79}Br)]$^+$ and a systematic shift for most fragment ions compared with the silyl derivative of **3**. In addition, the retention times of the pertrimethylsilyl derivatives of **4**, **5**, and **6** are shifted systematically in comparison to the pertrimethylsilyl derivatives of the chlorinated metabolites **1**, **2**, and **3**. Therefore, the arrangement of the substituents in each of the brominated compounds is presumably the same as in the corresponding chlorinated metabolites.

In addition, it was possible to isolate and purify small amounts of the phenol **7** by preparative and semipreparative HPLC from the cultures of *M. alcalina*, which had been supplemented with bromide. Compound **7** is 3-methoxy-5-methylbenzene-1,2-diol according to the EIMS, the ^{1}H NMR spectrum, and the GCEIMS of the pertrimethylsilyl derivative. The presence of two separated aromatic protons in the ^{1}H NMR with a $^4J_{HH}$ coupling (1.5 Hz) in the ^{1}H NMR spectrum implies that **7** is unsymmetrically substituted. Therefore, the methoxy group must be located *meta* to the methyl substituent. Phenol **7** differs from **1** and **4** only by the absence of the chloro and bromo substituents, respectively. Compound **7** was isolated from high-boiling wood tar oil 70 years ago.[7]

Since only traces of the phenol **8** were generated, it could be identified only by means of the GC-MS properties of its pertrimethylsilyl derivative.

Some chlorinated secondary metabolites have already been reported from the genus *Mycena*. These include mycenon,[8] tetrachloropyrocatechol,[4c] drosophilin A methyl ether,[9] and chlorinated benzoxepin[10] derivatives. Many chlorinated natural products have been isolated from other basidiomycetes and ascomycetes.[11] Chlorophenols usually exhibit antifungal activity[12] and protect the producer organism against competing species. However, there is no earlier report of the generation of brominated compounds from the genus *Mycena*. Reports of brominated natural products from fungi as a whole are rare.[13] 3-Bromo-4-methoxybenzaldehyde and 5-bromo-3,4-dimethoxybenzaldehyde have been identified by GC-MS in soil samples containing the mycelium of *Lepista nuda*.[14] A *Fusarium* sp. marine fungus from the Bahamas cultured in seawater-based medium has yielded the brominated sesterterpene neomangicol B as a minor constituent in addition to the corresponding chlorinated metabolite neomangicol A.[15] 2,3-Dibromo-4,5-dihydroxybenzyl alcohol has been isolated from a marine ascomycete cultured in a medium supplemented with potassium bromide.[16] CJ-19,784, a brominated antifungal flavone, has been isolated from cultures of an *Acanthostigmella* sp. fungus.[17] The white-rot fungus *Bjerkandera adusta* produces 3-chloro-4-methoxybenzaldehyde, 3-chloro-4-methoxybenzyl alcohol, and some more chlorinated volatile phenyl compounds.[18] Similarly to our experiments with *M. alcalina*, *B. adusta* generates the corresponding brominated metabolites after addition of bromide to the culture medium.[18] The substitution of chlorine by bromine sometimes also leads to a shift in the secondary metabolism. This was demonstrated in the case of the ascomycete *Lachnum papyraceum*, which produces several new nonbrominated compounds in addition to 4-bromo-6-hydroxymellein when calcium bromide is present in the culture medium.[19]

In the case of the alcalinaphenols A, B, D, and E, the halogenation is probably the last step in the biosynthesis, since the nonhalogenated phenol **7** could be detected as putative biosynthetic precursor of alcalinaphenols A and D and the nonhalogenated phenol **8** as putative precursor of alcalinaphenols B and E. Phenol **7** is an unsymmetrically substituted phenol, which could be halogenated in either position 4 or 6. However, the chlorination takes place only at the sterically more hindered C-4. Hence, the corresponding halogenating enzyme appears to operate regiospecifically. However, if bromide is added to the medium, the halogenase appears to brominate less selectively and less effectively, since unidentified dibrominated phenols are produced in trace amounts and the nonhalogenated phenols **7** and **8** are present in considerably larger quantities.

In general, two types of halogenating enzymes are known. The haloperoxidases, which are heme- or vanadium-dependent, generate HOX as halogenating agent from H_2O_2.[20] These haloperoxidases have been found in a variety of sources and usually exhibit a broad substrate specifity and a lack of regiospecifity.[21] The second class of halogenating enzymes are halogenases.[22] They are either $FADH_2$-dependent or nonheme α-ketoglutarate-dependent enzymes. The $FADH_2$-dependent enzymes require NADH, O_2, and a halogenide but not H_2O_2 for regioselective halogenation.[22] Nevertheless, halogenases also appear to tolerate bromide instead of chloride.[23] Probably, such a halogenase is also responsible for the production of the halogenated phenols in *M. alcalina*.

Experimental Section

General Experimental Procedures. Evaporation of the solvents was performed under reduced pressure using a rotary evaporator. Preparative HPLC: Waters 590EF pumps equipped with an automated gradient controller 680 and Kratos Spectroflow 783 UV-vis detector; column: Luna C-18 (2), 5 μm, 15 × 250 mm (Phenomenex); gradient: 10 min at 100% H_2O then within 30 min linear to 100% MeOH; flow rate: 6 mL min^{-1}; detection: UV at 220 nm. Semipreparative HPLC: Waters 510 pumps equipped with an automated gradient controller 680 and Applied Biosystems 783A UV-vis detector; column: Nucleodur C-18 EC, 5 μm, 10 × 250 mm (Macherey-Nagel); gradient: 10 min at 100% H_2O then within 30 min linear to 100% MeOH; flow rate: 6 mL min^{-1}; detection: UV at 220 nm. UV: Cary 100 Bio (Varian). NMR: Bruker DMX 600 spectrometer equipped with a cryoprobe (^{1}H at 600.13, ^{13}C at 150.9 MHz) and Bruker DMX 500 (^{1}H at 500.11, ^{13}C at 125.8 MHz), chemical shifts in δ relative to CD_3OD (δ_H 3.31, δ_C 49.00) as internal standard. EIMS: ThermoElectron Trace DSQ instrument equipped with direct insertion probe using EI at 70 eV. GCEIMS: ThermoElectron Trace DSQ coupled with a ThermoElectron Trace GC Ultra equipped with a PTV injector. For sample separation, a fused silica DB-5ms capillary column (15 m × 0.25 mm, coated with a 0.25 μm layer of liquid phase) and helium as carrier gas were used. Injection volumes were 0.2−0.5 μL of a 1−2% (m/v) solution of pertrimethylsilylated

crude extracts or purified samples. For pertrimethylsilylation *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was used. Temperature program: 1 min isothermal at 50 °C, then 5 K/min up to 300 °C, finally 10 min isothermal at 300 °C. Retention indices R_i according to Kováts[24] were determined by injection of a 0.2 μL sample of a standard mixture of saturated straight-chain alkanes (C_{10}−C_{36}). HREIMS: Finnigan-MAT 8200.

Fungal Material and Cultivation Conditions. Strain: CBS 386.90 (Centraalbureau voor Schimmelcultures, Amsterdam). Eighty petri dishes (90 mm diameter), each containing 25 g of solid MEA medium (30 g of malt extract, 5.0 g of peptone, 15 g of agar, 1000 mL of H_2O), were inoculated with mycelia of *M. alcalina* (CBS 386.90) and incubated for 8 weeks at 20 °C. Typically, the mycelia reached diameters of 5−6 cm. To test the specificity of the halogenase, *M. alcalina* was grown on MEA medium enriched with KBr (5.0 and 10.0 g/L, respectively). Under these conditions, the mycelia reached diameters of approximately 3−4 cm within 8 weeks.

Extraction and Isolation. The mycelia and inseparable parts of the medium were removed from 80 culture plates with a razor blade, yielding 70 g of crude material. The crude material was extracted with MeOH at 25 °C for 10 min. After filtration, the crude extract was concentrated in vacuum at 40 °C. The resulting residue was dissolved in 20 mL of MeOH/H_2O (1:1), prepurified with an RP-18 cartridge, and separated on an RP-18 column by preparative HPLC to afford 5-methyl-3-methoxycatechol (**7**), t_R 29.8 min; alcalinaphenol E (**5**), t_R 33.4 min; alcalinaphenol C (**3**), t_R 33.7 min; alcalinaphenol A (**1**), t_R 35.1 min; and alcalinaphenol B (**2**), t_R 35.5 min. The resulting alcalinaphenols **1**, **2**, **5**, and **7** were further purified by semipreparative HPLC to give 5-methyl-3-methoxycatechol (**7**), t_R 27.4 min; alcalinaphenol A (**1**), t_R 31.7 min; alcalinaphenol B (**2**), t_R 32.5 min; and alcalinaphenol E (**5**), t_R 32.9 min. Yields: Alcalinaphenol A (**1**), 0.84 mg; alcalinaphenol B (**2**), 0.44 mg; alcalinaphenol C (**3**), 0.60 mg; alcalinaphenol E (**5**), 0.66 mg; 5-methyl-3-methoxycatechol (**7**), 0.40 mg.

Alcalinaphenol A (4-chloro-3-methoxy-5-methylbenzene-1,2-diol, 1): colorless solid; UV (MeOH) λ_{max} 204, 283 nm; ^{1}H NMR (500 MHz, CD_3OD, 298 K) δ 6.50 (1H, s, H-6), 3.79 (3H, s, 3-OCH_3), 2.20 (3H, s, 5-CH_3); ^{13}C NMR (500 MHz, CD_3OD, 298 K) δ 145.96 (C, C-2), 145.75 (C, C-3), 138.56 (C, C-1), 127.49 (C, C-5), 119.01 (C, C-4), 113.66 (CH, C-6), 60.76 (CH_3, 3-OCH_3), 19.54 (CH_3, 5-CH_3); EIMS *m/z* 190 $[M(^{37}Cl)]^+$ (^{37}Cl) (35), 188 $[M(^{35}Cl)]^+$ (100), 175 $[M(^{37}Cl) - CH_3]^+$ (20), 173 $[M(^{35}Cl) - CH_3]^+$ (54), 147 $[M(^{37}Cl) - CH_3 - CO]^+$ (11), 145 $[M(^{35}Cl) - CH_3 - CO]^+$ (27); HREIMS *m/z* 188.02387 (calcd for $C_8H_9{}^{35}ClO_3$, 188.02402).

Pertrimethylsilyl derivative of 1: GCEIMS R_i 1656; *m/z* 334 $[M(^{37}Cl)]^+$ (22), 332 $[M(^{35}Cl)]^+$ (52), 319 $[M(^{37}Cl) - CH_3]^+$ (11), 317 $[M(^{35}Cl) - CH_3]^+$ (29), 304 $[M(^{37}Cl) - CH_2O]^+$ (12), 302 $[M(^{35}Cl) - CH_2O]^+$ (31), 289 $[M(^{37}Cl) - CH_2O - CH_3]^+$ (4), 287 $[M(^{35}Cl) - CH_2O - CH_3]^+$ (8), 251 $[M - CH_2O - CH_3 - Cl]^+$ (4), 244 (7), 201 (6), 193 (4), 179 (3), 166 (3), 151 (3), 93 (3), 75 $[(CH_3)_2SiOH]^+$ (5), 73 $[Si(CH_3)_3]^+$ (100), 59 (3), 45 (8).

Alcalinaphenol B (4-chloro-3,6-dimethoxy-5-methylbenzene-1,2-diol, 2): colorless solid; UV (MeOH) λ_{max} 205, 281 nm; ^{1}H NMR (600 MHz, CD_3OD, 300 K) δ 3.77 (3H, s, 3-OCH_3), 3.71 (3H, s, 6-OCH_3), 2.19 (3H, s, 5-CH_3); ^{13}C NMR (600 MHz, CD_3OD, 300 K) δ 144.11 (C, C-6), 142.09 (C, C-3), 120.57 (C, C-5), 118.44 (C, C-4), 60.58 (CH_3, 6-OCH_3), 60.57 (CH_3, 3-OCH_3), 12.44 (CH_3, 5-CH_3); EIMS *m/z* 220 $[M(^{37}Cl)]^+$ (15), 218 $[M(^{35}Cl)]^+$ (44), 205 $[M(^{37}Cl) - CH_3]^+$ (23), 203 $[M(^{35}Cl) - CH_3]^+$ (70), 190 $[M(^{37}Cl) - CH_2O]^+$ (32), 188 $[M(^{35}Cl) - CH_2O]^+$ (100), 175 (29), 173 (54), 145 (25); HREIMS *m/z* 218.03399 (calcd for $C_9H_{11}{}^{35}ClO_4$, 218.03459).

Pertrimethylsilyl derivative of 2: GCEIMS R_i 1713; *m/z* 364 $[M(^{37}Cl)]^+$ (24), 362 $[M(^{35}Cl)]^+$ (57), 349 $[M(^{37}Cl) - CH_3]^+$ (6), 347 $[M(^{35}Cl) - CH_3]^+$ (14), 334 $[M(^{37}Cl) - CH_2O]^+$ (40), 332 $[M(^{35}Cl) - CH_2O]^+$ (100), 319 (8), 317 (20), 304 (7), 302 (17), 274 (3), 267 (3), 261 (3), 259 (9), 216 (3), 151 (4), 133 (2), 89 (2), 75 (3), 73 $[Si(CH_3)_3]^+$ (42), 59 (3), 45 (6).

Alcalinaphenol C [3-chloro-2-(hydroxymethyl)-4,5,6-trimethoxyphenol, 3]: colorless solid; UV (MeOH) λ_{max} 206, 286 nm; ^{1}H NMR (500 MHz, CD_3OD, 298 K) δ 4.77 (2H, s, 2-CH_2OH), 3.91 (3H, s, 5-OCH_3), 3.84 (3H, s, 6-OCH_3 or 4-OCH_3), 3.78 (3H, s, 4-OCH_3 or 6-OCH_3); ^{13}C NMR (500 MHz, CD_3OD, 298 K) δ 148.24 (C, C-5), 147.49 (C, C-1), 143.47 (C, C-4 or C-6), 141.10 (C, C-6 or C-4), 124.44 (C, C-2), 121.46 (C, C-3), 61.32 (5-OCH_3), 61.27 (6-OCH_3 or 4-OCH_3), 61.22 (4-OCH_3 or 6-OCH_3), 57.17 (2-OCH_3); EIMS *m/z* 250 $[M(^{37}Cl)]^+$ (9), 248 $[M(^{35}Cl)]^+$ (22), 232 $[M(^{37}Cl) - H_2O]^+$ (38), 230 $[M(^{35}Cl) - H_2O]^+$ (100), 217 $[M(^{37}Cl) - H_2O - CH_3]^+$ (29), 215 $[M(^{35}Cl) - H_2O - CH_3]^+$ (65), 189 $[M(^{37}Cl) - H_2O - CH_3 - CO]^+$ (25), 187 $[M(^{35}Cl) - H_2O - CH_3 - CO]^+$ (60); HREIMS *m/z* 248.04529 (calcd for $C_{10}H_{13}{}^{35}ClO_5$, 248.04515).

Pertrimethylsilyl derivative of 3: GCEIMS R_i 1932; *m/z* 394 $[M(^{37}Cl)]^+$ (6), 392 $[M(^{35}Cl)]^+$ (18), 379 $[M(^{37}Cl) - CH_3]^+$ (4), 377 $[M(^{35}Cl) - CH_3]^+$ (12), 364 $[M(^{37}Cl) - CH_2O]^+$ (3), 362 $[M(^{35}Cl) - CH_2O]^+$ (9), 349 $[M(^{37}Cl) - CH_3 - CH_2O]^+$ (10), 347 $[M(^{35}Cl) - CH_3 - CH_2O]^+$ (20), 305 (6), 303 (11), 275 (6), 273 (10), 232 $[M(^{37}Cl) - (CH_3)_3SiOSi(CH_3)_3]^+$ (32), 230 $[M(^{35}Cl) - (CH_3)_3SiOSi(CH_3)_3]^+$ (100), 217 (17), 215 (36), 187 (29), 147 (30), 89 (5), 75 (9), 73 $[Si(CH_3)_3]^+$ (71), 59 (4), 45 (6).

Pertrimethylsilyl derivative of alcalinaphenol D (4-bromo-3-methoxy-5-methylbenzene-1,2-diol, 4): GCEIMS R_i 1735; *m/z* 378 $[M(^{81}Br)]^+$ (36), 376 $[M(^{79}Br)]^+$ (35), 363 $[M(^{81}Br) - CH_3]^+$ (19), 361 $[M(^{79}Br) - CH_3]^+$ (16), 348 $[M(^{81}Br) - CH_2O]^+$ (21), 346 $M(^{79}Br) - CH_2O]^+$ (20), 333 (7), 331 (6), 290 (4), 288 (4), 259 (11), 217 (11), 215 (9), 191 (9), 151 (4), 147 (15), 133 (4), 75 (7), 73 $[Si(CH_3)_3]^+$ (100), 45 (6).

Alcalinaphenol E (4-bromo-3,6-dimethoxy-5-methylbenzene-1,2-diol, 5): colorless solid; UV (MeOH) λ_{max} 210, 282; ^{1}H NMR (600 MHz, CD_3OD, 300 K) δ 3.76 (3H, s, 3-OCH_3), 3.71 (3H, s, 6-OCH_3), 2.23 (3H, s, 5-OCH_3); ^{13}C NMR (600 MHz, CD_3OD, 300 K) δ 144.27 (C, C-6), 143.00 (C, C-3), 139.78 (C, C-2), 138.91 (C, C-1), 122.26 (C, C-5), 109.10 (C, C-4), 60.56 (CH_3, 6-OCH_3), 60.40 (CH_3, 3-OCH_3), 15.35 (CH_3, 5-CH_3); EIMS *m/z* 264 $[M(^{81}Br)]^+$ (62), 262 $[M(^{79}Br)]^+$ (66), 249 $[M(^{81}Br) - CH_3]^+$ (96), 247 $[M(^{79}Br) - CH_3]^+$ (100), 221 $[M(^{81}Br) - CH_3 - CO]^+$ (25), 219 $[M(^{79}Br) - CH_3 - CO]^+$ (34), 206 $[M(^{81}Br) - CH_3 - COCH_3]^+$ (37), 204 $[M(^{79}Br) - CH_3 - COCH_3]^+$ (43).

Trimethylsilyl derivative of 5: GCEIMS R_i 1783; EIMS *m/z* 408 $[M(^{81}Br)]^+$ (27), 406 $[M(^{79}Br)]^+$ (28), 393 $[M(^{81}Br) - CH_3]^+$ (7), 391 $[M(^{79}Br) - CH_3]^+$ (7), 378 $[M(^{81}Br) - CH_2O]^+$ (50), 376 $[M(^{79}Br) - CH_2O]^+$ (46), 363 (11), 361 (10), 348 (10), 346 (10), 305 (4), 303 (5), 267 (3), 224 (3), 181 (4), 173 (3), 147 (4), 133 (4), 126 (4), 89 (4), 75 (7), 73 $[Si(CH_3)_3]^+$ (100), 59 (5), 45 (14).

Pertrimethylsilyl derivative of alcalinaphenol F [3-bromo-2-(hydroxymethyl)-4,5,6-trimethoxyphenol, 6]: GCEIMS R_i 2006; *m/z* 438 $[M(^{81}Br)]^+$ (3), 436 $[M(^{79}Br)]^+$ (3), 423 $[M(^{81}Br) - CH_3]^+$ (3), 421 $[M(^{79}Br) - CH_3]^+$ (2), 408 $[M(^{81}Br) - CH_2O]^+$ (2), 406 $[M(^{79}Br) - CH_2O]^+$ (2), 393 (6), 391 (5), 357 (2), 349 (3), 347 (2), 327 (2), 317 (3), 297 (2), 276 $[M(^{81}Br) - (CH_3)_3SiOSi(CH_3)_3]^+$ (31), 274 $[M(^{79}Br) - (CH_3)_3SiOSi(CH_3)_3]^+$ (33), 261 (12), 259 (13), 233 (12), 231 (11), 189 (5), 167 (3), 147 (29), 133 (5), 89 (6), 75 (16), 73 $[Si(CH_3)_3]^+$ (100), 59 (6), 45 (11).

3-Methoxy-5-methylbenzene-1,2-diol (7): colorless solid; UV (MeOH) λ_{max} 207, 273 nm; ^{1}H NMR (500 MHz, CD_3OD, 298 K) δ 6.29 (1H, d, ^4J = 1.5 Hz, H-4), 6.27 (1H, d, ^4J = 1.5 Hz, H-6), 3.80 (3H, s, 3-OCH_3), 2.19 (3H, s, 5-CH_3); EIMS *m/z* 154 $[M]^+$ (100), 139 $[M - CH_3]^+$ (65), 111 $[M - CH_3 - CO]^+$ (66), 71 (65).

Trimethylsilyl derivative of 7: GCEIMS R_i 1559; *m/z* 298 $[M]^+$ (19), 283 $[M - CH_3]^+$ (9), 268 $[M - CH_2O]^+$ (21), 253 $[M - CH_3 - CH_2O]^+$ (5), 210 (4), 167 (4), 147 (3), 133 (6), 119 (2), 75 (7), 73 (100), 59 (3), 45 (9).

Pertrimethylsilyl derivative of 3,6-dimethoxy-5-methylbenzene-1,2-diol (8): GCEIMS R_i 1615; *m/z* 328 $[M]^+$ (29), 313 $[M - CH_3]^+$ (7), 298 $[M - CH_2O]^+$ (52), 283 $[M - CH_3 - CH_2O]^+$ (5), 268 (19), 225 (8), 182 (10), 147 (6), 119 (5), 75 (17), 73 (100), 59 (7), 45 (21).

Acknowledgment. The work of P.S. has been supported by an Emmy-Noether Fellowship for young investigators of the Deutsche Forschungsgemeinschaft (SP 218/1-1) and by the Fonds der Chemischen Industrie.

Supporting Information Available: Selected NMR and mass spectra of compounds **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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NP0603368