Metabolites from the Wood-Rotting Basidiomycete *Hapalopilus mutans* (Aphyllophorales)

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Fruit bodies of the wood-rotting North American fungus *Hapalopilus mutans* produce a mixture of dihydroxybenzoic acids **1**, dihydroxybenzoquinones **2** and a novel type of spirodiones, e.g. mutadione A (**4a**). All compounds carry long alkyl chains, which may be partially unsaturated. The structures of the metabolites were established by spectroscopic methods.

The wood-rotting polypore *Hapalopilus mutans* (Pk.) Gilbn. & Ryv. is found in eastern North America from Canada to Florida.[1] It forms resupinate, soft fruit bodies on dead hardwoods, usually *Castanea*, and can cover large areas of the trunk. Interestingly, the creamy colour of the fruit bodies changes immediately to a reddish colour when bruised or treated with aqueous KOH. In this publication we report on the isolation and structural elucidation of the main metabolites of this fungus.

Results and Discussion

The air-dried and frozen fruit bodies of *Hapalopilus mutans* were extracted several times with methanol and the combined extracts concentrated to dryness. After partition of the residue between ethyl acetate and water, the components of the organic layer were separated by means of preparative HPLC on reversed-phase material. Diode-array UV detection revealed the presence of three different types of compounds: dihydroxybenzoic acids, benzoquinones and spirodiones in the approximate ratio of 5:1:20.

The structures of the dihydroxybenzoic acid derivatives **1a** and **1b** (Figure 1) were easily deduced from the ^{1}H- and ^{13}C-NMR data and the high resolution mass spectra. Weak [M^+] peaks are observed at *m*/*z* 388.2650 ($C_{24}H_{36}O_4$) and 390.2769 ($C_{24}H_{38}O_4$). Very strong [$M^+ - CO_2$] peaks at *m*/*z* 344.2715 ($C_{23}H_{36}O_2$) and 346.2867 ($C_{23}H_{38}O_2$) are indicative of carboxylic acids. The positions of the double bonds in the side chains were determined by ozonolysis. GC-MS analysis indicated the formation of hexanal and nonanal in the case of **1a** and **1b**, respectively. The ^{1}H- and ^{13}C-NMR data for the side-chain atoms of **1a** and **1b** agree well with those of linoleic acid and oleic acid, respectively.[2] The acids were therefore identified as 2-(heptadeca-8,11-dienyl)-4,6-dihydroxybenzoic acid ("dehydromerulinic acid A") (**1a**) and 2-(heptadec-8-enyl)-4,6-dihydroxybenzoic acid ($\Delta^{8'}$-merulinic acid A) (**1b**), which were isolated previously from *Phlebia radiata* and *Merulius tremellosus*.[3]

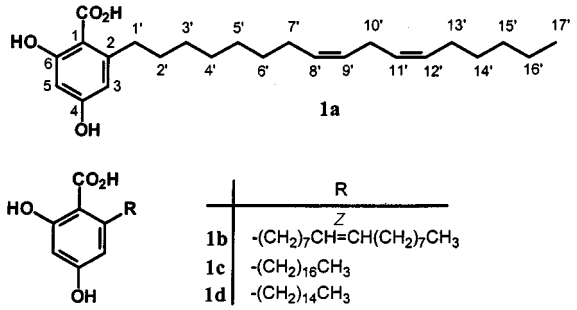

Figure 1. Dihydroxybenzoic acids **1** from *Hapalopilus mutans*

Two further dihydroxybenzoic acid derivatives could not be completely separated by HPLC. From the HR-EIMS, UV and ^{1}H-NMR spectra, one component was identified as 2-heptadecyl-4,6-dihydroxybenzoic acid ("dihydromerulinic acid A")[3] (**1c**), whereas a second component, 2,4-dihydroxy-6-pentadecylbenzoic acid ("corticiolic acid") (**1d**), was obtained only in admixture with **1b**. **1d** has already been reported as a constituent of *Corticium caeruleum* (Aphyllophorales).[4]

The second group of compounds are the dihydroxy-1,4-benzoquinones **2a-d**, which have different side chains at C-2 (Figure 2). The HR-EIMS of the main component **2a** showed a molecular ion peak at *m*/*z* 374.2456 corresponding to the molecular formula $C_{23}H_{34}O_4$. However, in the ^{13}C-NMR spectrum only 19 carbon signals could be observed due to rapid tautomerization of the 3,6-dihydroxy-1,4-benzoquinone system.[5] The dimethyl ether **3** derived from **2a** by treatment with diazomethane exhibited 25 carbon signals in the ^{13}C-NMR spectrum and showed the expected [M^+] peak at *m*/*z* 402.2785 ($C_{25}H_{38}O_4$). The position of the double bonds in the side chain follows from the close correspondence of the ^{1}H- and ^{13}C-NMR signals with those of acid **1a**.

The quinone **2b** was obtained nearly pure, but the minor components **2c** and **2d** were an inseparable mixture. Their formulas were deduced from the UV, NMR and HR-MS data and by comparison with the data of the corresponding acids **1**. The structures of the side chains point to a close biosynthetic relationship with the dihydroxybenzoic acid

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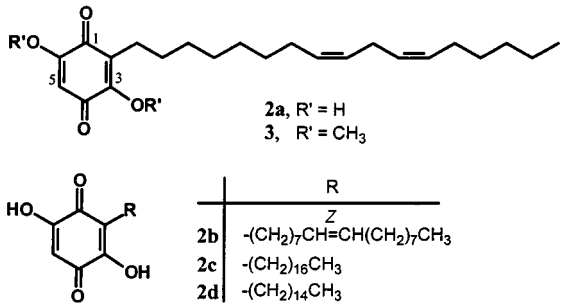


Figure 2. Dihydroxy-1,4-benzoquinones **2** from *Hapalopilus mutans*

derivatives. Obviously, the benzoquinones are present in the fruit bodies as their leuco derivatives and are oxidized to the quinones on bruising. The 2,5-dihydroxy-1,4-benzoquinones **2a,b** and **2d** have been isolated before from plants of the Myrsinaceae[6−8] and Connaraceae[9] families.

The third group of compounds includes several substances with a novel spirodione structure, for which we propose the name mutadione (Figure 3). The structural elucidation of these compounds was hampered by the small number of hydrogen atoms attached to the core structure of the molecule. The major component, named mutadione A (**4a**), could be obtained pure, whereas several other compounds of the same type were inseparable mixtures. The HR-EIMS of mutadione A exhibited an [M^+] ion peak at *m/z* 730.4814, corresponding to $C_{46}H_{66}O_7$. This suggested that mutadione A is formed by condensation of two molecules of benzoquinone **2a** (M = 374) with removal of one molecule of water (2 × 374 − 18 = 730).

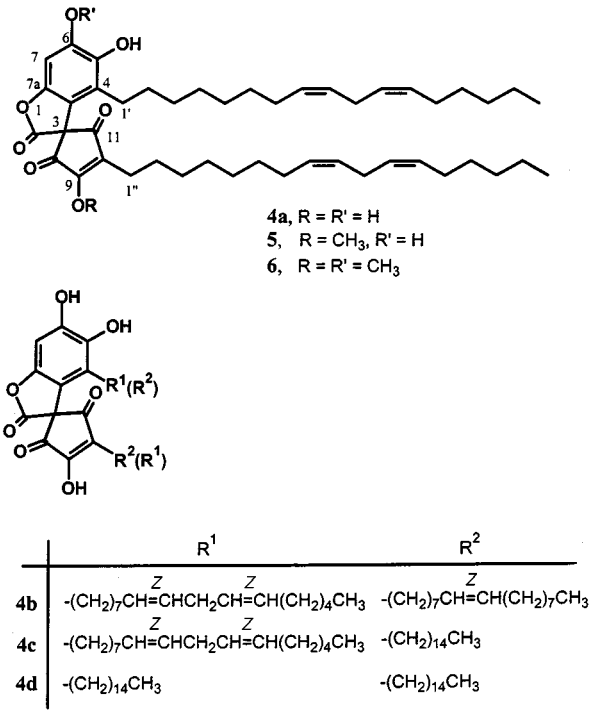


Figure 3. Mutadiones **4** from *Hapalopilus mutans*

In the ^{1}H-NMR spectrum, a singlet at δ = 6.26 corresponds to an aromatic CH group (δ_C = 97.0) flanked by two *O*-functions in the *ortho* positions. A multiplet at δ = 5.35 can be assigned to 8 olefinic CH groups from two linoleic acid chains attached to the central unit. In the ^{13}C-NMR spectrum, 46 signals were observed, 12 of which belong to the core structure. The signals at δ = 193.3 and 191.9 can be assigned to α,β-unsaturated ketone functions and the signal at δ = 64.5 to a quaternary C atom. The observed chemical shifts compare well with those for synthetic compounds of this type.[10][11] The structure of mutadione A (**4a**) could finally be solved by a careful analysis of its HMQC, HMBC (Figure 4) and proton-coupled ^{13}C-NMR spectra.

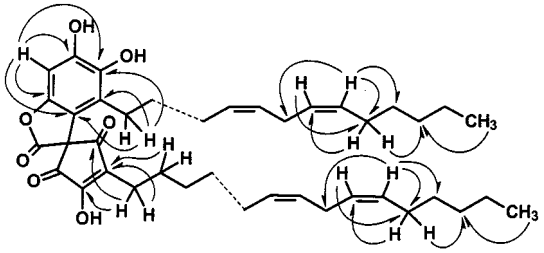


Figure 4. Selected HMBC correlations for mutadione A (**4a**)

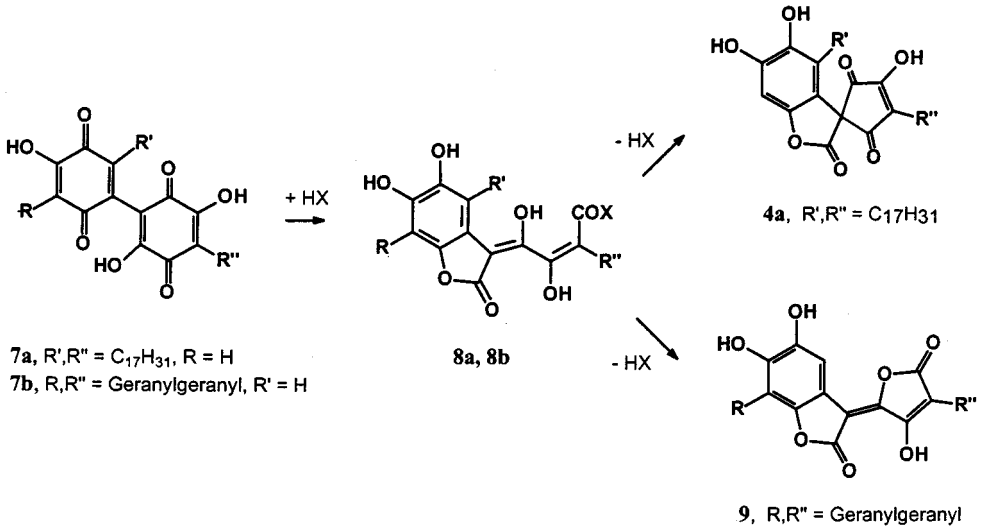
With one equivalent of diazomethane mutadione A (**4a**) yields the 9-*O*-methyl ether **5**. Excess of the reagent leads to the 6,9-*O*-dimethyl ether **6** which exhibits an NOE between the methoxy group at C-6 and the aromatic proton at C-7.

In addition to mutadione A (**4a**) we have isolated as minor constituents the mutadiones B (**4b**), C (**4c**) and D (**4d**). In the case of compounds **4b** and **4c** the relative positions of the side chains could not be determined. There is the possibility that the two regioisomers are present in each case.

The mutadiones exhibit neither optical rotations nor CD effects, in spite of the presence of the stereogenic spiro center. On addition of an equimolar amount of the chiral shift reagent tris[3-trifluoromethylhydroxymethylene-(+)-camphorato]europium(III) [Eu(tfc)$_3$] to a solution of **4a** in $CDCl_3$, a 1:1 splitting of the ^{1}H-NMR signals is observed, in accord with the presence of a racemic mixture. The signal of the aromatic proton is thereby shifted from δ = 6.26 to δ = 6.61 and 7.01.

A biosynthesis of mutadione A (**4a**) can be proposed (Scheme 1), which resembles that of bovilactone-4,4 (**9**) from *Suillus bovinus*.[12] In both cases a diquinone of type **7** can be considered as precursor which suffers ring cleavage with concomitant lactone formation,[13−15] to yield an enol intermediate **8**. In the case of **8a** intramolecular *C*-acylation with formation of the spirodione **4a** can take place, whereas in the case of **8b** *O*-acylation affords the dilactone **9**.

Interestingly, in mycelial culture on Moser minimal medium[16] *H. mutans* produces mainly benzoquinone **2a** and small amounts of **2b** and **2d**.[17] Whereas traces of 2,4-dihydroxybenzoic acids **1** could be detected by HPLC, the mutadiones **4** were not found.



Scheme 1. Proposal for the formation of mutadione A (**4a**) and boviquinone-4,4 (**9**) from the precursors **7a** and **7b**, respectively

Experimental Section

General: Melting points (uncorrected): Reichert hot stage. − Optical rotations: Perkin-Elmer 214. − UV/Vis: Perkin-Elmer Lambda 16. − IR: Perkin-Elmer FT-IR 1000. − NMR: Bruker ARX-300, AMX-600 and Varian VXR-400 S, in $CDCl_3$ or CD_3OD, solvent peak as internal reference. − MS: Finnigan MAT 90 and MAT 95Q. − GC-MS was performed with a Varian GC 3200 coupled with a Finnigan MAT Magnum mass spectrometer (ion trap). Column: J & W DB-5ms, length 30 m, diameter 0.25 mm. Temperature gradient: 50°C for 2 min, then heating with 5°C/min up to 300°C. − TLC: Silica gel Merck Kieselgel 60 F_{254} plates (thickness 0.2 mm); solvent system: toluene/HCO_2Et/HCO_2H (10:5:3). The brown or yellow spots gave distinct colours on spraying with a mixture of 4-methoxybenzaldehyde/AcOH/H_2SO_4/EtOH (10:12:12:210) (ANIS) and heating. − Solid-phase extractions: Chromabond C18 cartridges (Macherey-Nagel). − Analytical HPLC (system 1): Waters 600 E Pump and System Controller with Diode-Array Detector 990+; Knauer Vertex column 4 ± 250 mm, packed with Nucleosil 100 C18, 5 μm; eluent A: H_2O/CH_3CN (9:1) + 0.5% CF_3CO_2H; eluent B: CH_3CN; linear gradient: 0 min: A 100%, 30 min: B 100%; flow rate 1 mL/min, detection range 200−400 nm. − Preparative HPLC (system 2): Merck Hitachi L 6200 Intelligent Pump and 655A Variable Wavelength UV Monitor. Knauer Vertex columns 16 ± 250 mm, packed with Nucleosil 100 C18, 7 μm; eluents as for analytical HPLC; linear gradient: 0 min: A 20%, B 80%, 40 min: B 100%; flow rate 7 mL/min, detection at 290 nm.

Biological Material: Hapalopilus mutans was collected in August 1989, 1994 and 1997 from a dead *Castanea dentata* tree in the Botanical Garden of the Biological Station in Highlands, NC, USA. Part of the material was air-dried and than stored at −10°C, while the remainder was immediately frozen on collection. A voucher specimen is kept in the herbarium of the Ludwig-Maximilians-Universität München, Institut für Organische Chemie.

Isolation of the Metabolites from the Fruit Bodies (Typical Work-up): 100 g of fresh fruit bodies of *H. mutans* were extracted several times with 500 mL of methanol until the resulting solution was colourless. After evaporation of the solvent, the brown, oily residue was partitioned between ethyl acetate and water. The organic layers were dried ($MgSO_4$) and the solvent removed in vacuo. The oily residue was dissolved in methanol and the mixture separated by reversed-phase preparative HPLC (C-18 material) with an acidified water/acetonitrile gradient (system 2).

2-(Heptadeca-8,11-dienyl)-4,6-dihydroxybenzoic Acid, Dehydromerulinic Acid A (1a): 10 mg (0.01%) of a slightly yellow amorphous powder, m.p. 90−92°C. − TLC: R_f = 0.57, red spot with ANIS. − HPLC (system 2): t_R = 17.33 min. − UV (CH_3OH): λ_{max} (log ε) = 217 (4.69), 263 (4.37), 301 (3.98) nm. − IR ($CHCl_3$, film): $\tilde{\nu}$ = 3400 cm^{-1}(br. m), 2927 (s), 2855 (s), 1623 (s), 1465 (m), 1367 (m), 1258 (s), 1170 (m), 1106 (w), 1021 (w), 849 (w), 724 (w), 619 (w). − ^1H NMR (300 MHz, CD_3OD): δ = 0.97 (t, *J* = 6 Hz, 3 H, 17′-H), 1.40 (m, 14 H), 1.64 (m, 2 H, 2′-H), 2.14 (m, 4 H, 7′-, 13′-H), 2.85 (t, *J* = 6 Hz, 2 H, 10′-H), 2.97 (t, *J* = 7 Hz, 2 H, 1′-H), 5.42 (m, 4 H, 8′-, 9′-, 11′-, 12′-H), 6.20 (d, *J* = 2 Hz, 1 H, 5-H), 6.25 (d, *J* = 2 Hz, 1 H, 3-H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1 (q, C-17′), 22.6 (t), 25.6 (t, C-10′), 27.2 (t), 27.2 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.6 (t), 29.6 (t), 29.7 (t), 31.5 (t), 31.7 (t), 101.5 (d, C-5), 103.6 (d, C-3), 111.1 (s, C-1), 127.9 (d, C-11′), 128.0 (d, C-9′), 130.1 (d, C-8′), 130.2 (d, C-12′), 150.4 (s, C-2), 161.3 (s, C-6), 166.4 (s, C-4), 174.7 (s, COOH). − EI MS (DI, 220°C, 70 eV); *m/z* (%): 388 (1) [M^+], 344 (18) [$M^+ - CO_2$], 163 (14), 137 (12), 124 (100), 123 (27). − $C_{24}H_{36}O_4$: calcd. 388.2614; found 388.2650 (HR EIMS).

2-(Heptadec-8-enyl)-4,6-dihydroxybenzoic Acid, Merulinic Acid A (1b): 10 mg (0.01%) of a slightly yellow amorphous powder, m.p. 95−97°C. − TLC: R_f = 0.57, red spot with ANIS. − HPLC (system 2): t_R = 22.90 min. − UV (CH_3OH): λ_{max} (log ε) = 217 (4.35), 262 (4.03), 300 (3.67) nm. − IR ($CHCl_3$, film): $\tilde{\nu}$ = 3392 cm^{-1}(br. m), 2923 (s), 2852 (s), 1624 (s), 1467 (m), 1361 (m), 1251 (s), 1201 (w), 1177 (w), 1025 (w), 624 (w). − ^1H NMR (300 MHz, $CDCl_3$): δ = 0.87 (t, *J* = 7 Hz, 3 H, 17′-H), 1.30 (m, 20 H), 1.57 (m, 2 H, 2′-H), 2.00 (m, 4 H, 7′-, 13′-H), 2.90 (t, *J* = 7 Hz, 2 H, 1′-H), 5.35 (t, *J* = 5 Hz, 2 H, 8′-, 9′-H), 6.27 (m, 2 H, 3-,5-H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1 (q, 17′-H), 22.7 (t), 22.7 (t), 27.2 (t), 27.2 (t), 29.3 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 29.8 (t), 31.8 (t), 31.9 (t), 101.4 (d, C-5), 103.6 (s, C-1), 111.2 (d, C-3), 129.8 (d, C-8′), 130.0 (d, C-9′), 150.4 (s, C-2), 161.3 (s, C-6), 166.3 (s, C-4), 174.6 (s, COOH). − EI MS (DI, 220°C, 70 eV); *m/z* (%): 390 (0.1) [M^+], 346 (4) [$M^+ - CO_2$], 320 (10), 168 (5), 166 (4), 137 (10), 125 (7), 124 (100), 123 (15). − $C_{24}H_{38}O_4$: calcd. 390.2768; found 390.2769 (HR EIMS).

Ozonolysis of the Acids 1a and 1b: Through solutions of 1 mg of either **1a** or **1b** in 5 mL of CH_2Cl_2 a stream of ozone was passed at −78°C, until the colour of the solutions remained slightly violet. The excess of O_3 was removed by bubbling O_2 through the reaction mixtures. After reductive work-up by addition of dimethyl sulfide (0.5 mL) and GC MS of the resulting reaction mixtures, hexanal (*m/z* 101 [M^+ + H], R_t = 2.23 min) was detected in the case of **1a** and nonanal (*m/z* 143 [M^+ + H], R_t = 9.55 min) in the case of **1b**.

2-Heptadecyl-4,6-dihydroxybenzoic Acid, Dihydromerulinic Acid A (1c): 6 mg (0.006%) of a slightly yellow amorphous powder, m.p. 105−107°C. − TLC: R_f = 0.57, red spot with ANIS. − HPLC (system 2): t_R = 31.80 min. − UV (CH_3OH): λ_{max} (log ε) = 217 (3.80), 262 (3.47), 297 (3.16) nm. − IR ($CHCl_3$,film): $\tilde{\nu}$ = 3380 cm^{-1}(br. m), 2920 (s), 2850 (s), 1625 (s), 1469 (m), 1361 (m), 1254 (m), 1200 (m), 1179 (m), 1028 (w), 766 (w), 624 (w). − ^{1}H NMR (300 MHz, CD_3OD): δ = 0.98 (t, *J* = 7 Hz, 3 H, 17′-H), 1.37 (m, 28 H), 1.63 (m, 2 H, 2′-H), 2.97 (t, *J* = 7 Hz, 2 H, 1′-H), 6.22 (d, *J* = 2 Hz, 1 H, 5-H), 6.27 (d, 2 Hz, 1 H, 3-H). − EI MS (DI, 220°C, 70 eV); *m/z* (%): 392 (2) [M^+], 349 (11) [$M^+ - CO_2$], 348 (46), 166 (5), 137 (10), 125 (7), 124 (100), 123 (21). − $C_{24}H_{40}O_4$: calcd. 392.2926; found 392.2928 (HR EIMS).

2,4-Dihydroxy-6-pentadecylbenzoic Acid, Corticiolic Acid (1d): < 1 mg of a slightly yellow amorphous powder, which could only be isolated in admixture with **1b**. − TLC: R_f = 0.57, red spot with ANIS. − HPLC (system 2): t_R = 22.90 min. − UV (CH_3OH): λ_{max} (Abs_{rel}) = 217 (100), 262 (80), 300 (70). − ^{1}H NMR (300 MHz, CD_3OD): δ = 0.97 (t, *J* = 7 Hz, 3 H, 15′-H), 1.37 (m, 24 H), 1.64 (m, 2 H, 2′-H), 2.96 (t, *J* = 8 Hz, 2 H, 1′-H), 6.22 (d, *J* = 2 Hz, 1 H, 3-H), 6.27 (d, *J* = 2 Hz, 1 H, 5-H). − EI MS (DI, 220°C, 70 eV); *m/z* (%): 364 (0.02) [M^+], 320 (4) [$M^+ - CO_2$], 124 (100). − $C_{22}H_{36}O_4$: calcd. 364.2613; found 364.2645 (HR EIMS).

2-(Heptadeca-8,11-dienyl)-3,6-dihydroxy-1,4-benzoquinone (2a): 5 mg (0.005%) of a slightly yellow amorphous powder, m.p. 93−95°C. − TLC: R_f = 0.66, blue spot with ANIS. − HPLC (system 2): t_R = 22.00 min. − UV (CH_3OH): λ_{max} (log ε) = 208 (3.89), 288 (4.05), 428 (2.21) nm. − IR (KBr): $\tilde{\nu}$ = 3430 cm^{-1}(br. w), 3306 (s), 2957 (sh), 2925 (s), 2854 (m), 1636 (sh), 1617 (s), 1333 (s), 1190 (m), 860 (w), 768 (w), 704 (w). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.87 (t, *J* = 7 Hz, 3 H, 17′-H), 1.31 (m, 14 H), 1.47 (m, 2 H, 2′-H), 2.04 (m, 4 H, 7′-, 13′-H), 2.45 (t, *J* = 8 Hz, 2 H, 1′-H), 2.77 (t, *J* = 6 Hz, 2 H, 10′-H), 5.35 (m, 4 H, 8′-, 9′-, 11′-, 12′-H), 6.00 (s, 1 H, 5-H), 7.67 (s, 2 H, 2 × OH). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1 (q, C-17′), 22.5 (t), 22.6 (t), 25.6 (t, C-10′), 27.2 (t), 27.2 (t), 27.9 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 31.5 (t), 102.2 (d, C-5), 116.9 (s, C-2), 127.9 (d, C-11′), 128.0 (d, C-9′), 130.1 (d, C-8′), 130.2 (d, C-12′). − EI MS (DI, 220°C, 70 eV); *m/z* (%): 374 (64) [M^+], 360 (5), 356 (4), 348 (5), 280 (9), 266 (4), 252 (7), 238 (9), 155 (78), 154 (100), 153 (21), 95 (31). − $C_{23}H_{34}O_4$: calcd. 374.2457; found 374.2456 (HR EIMS).

2-(Heptadeca-8,11-dienyl)-3,6-dimethoxy-1,4-benzoquinone (3): A solution of CH_2N_2 in diethyl ether at 0°C was added dropwise to a solution of 4.2 mg (0.01 mmol) of **2a** in 3 mL methanol. The reaction was monitored by analytical HPLC. After addition of 6 mL of the CH_2N_2 solution, no more starting material was detectable and the reaction mixture was concentrated to dryness giving the analytically pure dimethyl ether **3**. Yield: 4.4 mg (0.01 mmol, 98%) of a brownish oil. − TLC: R_f = 0.73, blue spot with ANIS. − UV (CH_3OH): λ_{max} (log ε) = 208 (3.89), 230 (3.68), 283 (3.74). − IR ($CHCl_3$, film): $\tilde{\nu}$ = 3468 cm^{-1} (br. w), 3008 (w), 2927 (s), 2854 (s), 1742 (w), 1656 (s), 1599 (s), 1459 (m), 1326 (m), 1212 (s), 1156 (w), 1046 (m), 844 (w), 722 (w). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.89 (t, *J* = 7 Hz, 3 H, 17′-H), 1.30 (m, 14 H), 1.56 (m, 2 H, 2′-H), 2.04 (m, 4 H, 7′-, 13′-H), 2.43 (t, *J* = 8 Hz, 2 H, 1′-H), 2.77 (t, *J* = 6 Hz, 2 H, 10′-H), 3.80 (s, 3 H, 6-OCH_3), 4.05 (s, 3 H, 3-OCH_3), 5.35 (m, 4 H, 8′-, 9′-, 11′-, 12′-H), 5.73 (s, 1 H, 5-H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1 (q, C-17′), 22.5 (t), 23.1 (t), 25.6 (t, C-10′), 27.2 (t, 2C), 28.7 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 31.5 (t), 56,3 (q, OCH_3), 61.2 (q, OCH_3), 105.4 (d, C-5), 127.9 (d, C-11′), 128.0 (d, C-9′), 128.6 (s, C-2), 130.1 (d, C-8′), 130.2 (d, C-12′), 155.9 (s, C-3), 158.8 (s, C-6), 182.4 (s, C-1), 183.6 (s, C-4). − EI MS (DI, 220°C, 70 eV); *m/z* (%): 402 (23) [M^+], 196 (13), 183 (40), 169 (37), 153 (21), 123 (24), 97 (17), 95 (16). − $C_{25}H_{38}O_4$: calcd. 402.2771; found 402.2785 (HR EIMS).

2-(Heptadec-8-enyl)-3,6-dihydroxy-1,4-benzoquinone (2b): < 1 mg of a slightly yellow amorphous powder. − TLC: R_f = 0.66, blue spot with ANIS. − HPLC (system 2): t_R = 27.08 min. − UV (CH_3OH): λ_{max} (log ε) = 289 (3.46) nm. − IR ($CHCl_3$, film): $\tilde{\nu}$ = 3306 cm^{-1}(s), 2955 (s), 2850 (s), 1697 (m), 1615 (s), 1465 (m), 1356 (s), 1331 (s), 1193 (s), 837 (w), 768 (w), 707 (w). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3 H, 17′-H), 1.30 (m, 20 H), 1.55 (m, 2 H, 2′-H), 2.00 (m, 4 H, 7′-, 13′-H), 2.45 (t, *J* = 7 Hz, 2 H, 1′-H), 5.34 (t, *J* = 7 Hz, 2 H, 8′-, 9′-H), 6.00 (s, 1 H, 5-H), 7.66 (s, 2 H, 2 × OH). − EI MS (DI, 220°C, 70 eV); *m/z* (%): 376 (23) [M^+], 155 (90), 154 (100). − $C_{23}H_{36}O_4$: calcd. 376.2614; found 376.2612 (HR EIMS).

2-(Heptadecyl)-3,6-dihydroxy-1,4-benzoquinone (2c): < 1 mg of a slightly yellow amorphous powder, which could only be detected in the EIMS as a mixture with **2a** and **2d**. − TLC: R_f = 0.66, blue spot with ANIS. − $C_{23}H_{38}O_4$: calcd. 378.2770; found 378.2767 (HR EIMS).

2,5-Dihydroxy-3-pentadecyl-1,4-benzoquinone (2d): < 1 mg of a slightly yellow amorphous powder, which could only be detected in the EIMS as a mixture with **2b**. − TLC: R_f = 0.66, blue spot with ANIS. − HPLC (system 2): t_R = 27.08 min. − UV (MeOH): λ_{max} (abs.) = 289 (100) nm. − $C_{21}H_{34}O_4$: calcd. 350.2457; found 350.2456 (HR EIMS).

Mutadione A (4a): 100 mg (0.1%) of a brownish oil. − TLC: R_f = 0.53, blue spot with ANIS. − HPLC (system 2): t_R = 50.87 min. − UV ($CHCl_3$): λ_{max} (log ε) = 287 (4.07) nm. − IR ($CHCl_3$, film): $\tilde{\nu}$ = 3410 cm^{-1} (br. m), 3008 (m), 2925 (s), 2853 (s), 1785 (m), 1773 (m), 1741 (m), 1690 (s), 1637 (s), 1603 (w), 1483 (m), 1467 (m), 1385 (s), 1308 (m), 1222 (w),1082 (m), 1015 (w), 831 (w). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.88 (m, 6 H, 17′-, 17′′-H), 1.18−1.40 (m, 30 H), 1.63 (m, 2 H, 2′′-H), 2.05 (m, 8 H, 7′-, 7′′-, 13′-, 13′′-H), 2.13 (m, 2 H, 1′-H), 2.56 (t, *J* = 8 Hz, 2 H, 1′′-H), 2.77 (m, 4 H, 10′-, 10′′-H), 5.35 (m, 8 H, 8′-, 8′′-, 9′-, 9′′-, 11′-, 11′′-, 12′-, 12′′-H), 6.26 (s, 1 H, 7-H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1 (q, 2C, C-17′, -17′′), 22.6 (t, 2C), 22.7 (t), 25.6 (t, 2C, C-10′, -10′′), 27.0 (t), 27.1 (t, 2C), 27.2 (t, 2C), 28.5 (t), 28.9 (t), 29.1 (t), 29.20 (t, 2C), 29.3 (t), 29.4 (t), 29.6 (t), 29.7 (t, 2C), 29.9 (t), 31.5 (t), 31.9 (t), 64.5 (s, C-3), 97.0 (d, C-7), 111.8 (s, C-3a), 126.6 (s, C-4), 127.8 (d), 127.9 (d), 128.0 (d), 128.1 (d), 129.9 (d), 130.0 (d), 130.2 (d), 130.3 (d), 139.8 (s, C-10), 139.9 (s, C-5), 145.2 (s, C-6), 147.9 (s, C-7a), 167.6 (s, C-9), 169.3 (s, C-2), 191.8 (s, C-8), 193.3 (s, C-11). − EI MS (DI, 220°C, 70 eV); *m/z* (%): 730 (100) [M^+], 716 (13), 705 (13), 704 (27), 690 (24), 660 (19), 659 (15), 646 (16), 645 (27), 619 (13), 402 (16), 401 (14), 400 (47), 376 (14), 374 (15), 289 (36), 219 (24), 205 (26), 179 (36), 95 (60), 83 (24), 69 (45), 67 (72), 55 (62). − $C_{46}H_{66}O_7$: calcd. 730.4808; found 730.4814 (HR EIMS).

Methylation of Mutadione A: A solution of CH_2N_2 in diethyl ether at 0°C was added dropwise to a solution of 32 mg (0.04 mmol) of **4a** in 5 mL of CH_3OH and 5 mL of CH_3CN. The reaction was monitored by analytical HPLC. After addition of 15 mL of the

CH_2N_2 solution, no more **4a** was detectable and the reaction mixture was concentrated to dryness yielding analytically pure mutadione A 9-*O*-methyl ether (**5**). Addition of 12 mL of CH_2N_2 solution to 20 mg (0.03 mmol) of **4a** in 5 mL of CH_3OH afforded mutadione A 6,9-di-*O*-methyl ether (**6**).

5: Yield 32 mg (0.04 mmol, 98%) of a brownish oil. − TLC: R_f = 0.61, blue spot with ANIS. − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.89 (m, 6 H, 17′-, 17′′-H), 1.18−1.40 (m, 30 H), 1.57 (m, 2 H, 2′′-H), 2.05 (m, 8 H, 7′-, 7′′-, 13′-, 13′′-H), 2.19 (m, 2 H, 1′-H), 2.51 (t, J = 8 Hz, 2 H, 1′′-H), 2.77 (m, 4 H, 10′-, 10′′-H), 4.37 (s, 3 H, 9-OCH_3), 5.35 (m, 8 H, 8′-, 8′′-, 9′-, 9′′-, 11′-, 11′′-, 12′-, 12′′-H), 6.41 (s, 1 H, 7-H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.0 (q, 2C, C-17′, -17′′), 22.5 (t, 2C), 22.7 (t), 25.6 (t, C-10′, -10′′), 26.2 (t), 27.2 (t, 4C), 27.3 (t), 28.4 (t), 28.8 (t), 29.1 (t, 2C), 29.20 (t), 29.3 (t, 2C), 29.5 (t), 29.6 (t), 29.7 (t), 29.9 (t), 31.5 (t, 2C), 60.4 (q, 9-OCH_3), 66.3 (s, C-3), 97.0 (d, C-7), 111.8 (s, C-3a), 126.3 (s, C-4), 127.9 (d, 2C), 128.0 (d, 2C), 130.0 (d, 2C), 130.2 (d, 2C), 140.0 (s, C-5), 144.1 (s, C-10), 148.0 (s, C-7a), 145.4 (s, C-6), 168.1 (s, C-9), 169.2 (s, C-2), 190.3 (s, C-8), 192.1 (s, C-11). − EI MS (DI, 220°C, 70 eV); *m*/*z* (%): 744 (79) [M^+], 732 (74), 720 (30), 718 (44), 702 (14), 95 (72), 81 (86), 67 (100). − $C_{47}H_{68}O_7$: calcd. 744.4965; found 744.4966 (HR EIMS).

6: Yield 19.5 mg (0.02 mmol, 92%) of a brownish oil. − TLC: R_f = 0.68, blue spot with ANIS. − UV (CH_3OH): λ_{max} (log ε) = 219 (4.23), 286 (3.89) nm. − IR ($CHCl_3$, film): $\tilde{\nu}$ = 3418 cm^{-1} (br. m), 3009 (w), 2927 (s), 2855 (s), 1799 (m), 1742 (m), 1694 (s), 1614 (m), 1478 (m), 1460 (m), 1442 (m), 1350 (m), 1267 (w), 1226 (w), 1106 (m), 1017 (w), 818 (w), 724 (w). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (m, 6 H, 17′-, 17′′-H), 1.18−1.40 (m, 30 H), 1.57 (m, 2 H, 2′′-H), 2.05 (m, 8 H, 7′-, 7′′-, 13′-, 13′′-H), 2.19 (m, 2 H, 1′-H), 2.51 (t, J = 8 Hz, 2 H, 1′′-H), 2.76 (m, 4 H, 10′-, 10′′-H), 3.90 (s, 3 H, 6-OCH_3), 4.35 (s, 3 H, 9-OCH_3), 5.35 (m, 8 H, 8′-, 8′′-, 9′-, 9′′-, 11′-, 11′′-, 12′-, 12′′-H), 6.64 (s, 1 H, 7-H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.0 (q, 2C, C-17′, -17′′), 22.5 (t, 2C), 22.7 (t), 25.6 (t, C-10′, -10′′), 26.2 (t), 27.2 (t, 4C), 27.3 (t), 28.4 (t), 28.8 (t), 29.1 (t, 2C), 29.20 (t), 29.3 (t, 2C), 29.5 (t), 29.6 (t), 29.7 (t), 29.9 (t), 31.5 (t, 2C), 56.4 (q, 6-OCH_3), 60.3 (q, 9-OCH_3), 66.3 (s, C-3), 93.3 (d, C-7), 112.8 (s, C-3a), 125.6 (s, C-4), 127.9 (d, 2C), 128.0 (d, 2C), 130.0 (d, 2C), 130.2 (d, 2C), 140.9 (s, C-5), 144.1 (s, C-10), 147.9 (s, C-7a), 148.1 (s, C-6), 167.7 (s, C-9), 169.1 (s, C-2), 190.1 (s, C-8), 190.7 (s, C-11). − EI MS (DI, 220°C, 70 eV); *m*/*z* (%): 758 (100) [M^+], 746 (25), 732 (40), 414 (5), 193 (9), 95 (21), 81 (24), 67 (27). − $C_{48}H_{70}O_7$: calcd. 758.5121; found 758.5115 (HR EIMS).

Mixture of Mutadione B (4b) and Mutadione C (4c): 25 mg (0.025%) of a brownish oil. − TLC: R_f = 0.53, blue spot with ANIS. − HPLC (system 2): t_R = 67.22 min. − UV (CH_3CN): λ_{max} ($abs._{rel}$) = 219 (100), 285 (71) nm. − IR ($CHCl_3$, film): $\tilde{\nu}$ = 3415 cm^{-1}(br. m), 3010 (m), 2924 (s), 2853 (s), 1787 (s), 1742 (m), 1690 (s), 1639 (s), 1483 (m), 1466 (m), 1386 (s), 1309 (m), 1223 (w), 1125 (w), 1085 (m), 1015 (w), 831 (w), 722 (w). − EI MS (DI, 220°C, 70 eV); *m*/*z* (%): 732 (33) [**4b**-M^+], 706 (31) [**4c**-M^+], 692 (27), 667 (20), 666 (43), 402 (81), 377 (25), 376 (100), 303 (24), 289 (50), 192 (26), 179 (47), 95 (44), 81 (48), 69 (40). − **4b**: $C_{46}H_{68}O_7$: calcd. 732.4965; found 732.4924; **4c**: $C_{44}H_{66}O_7$: calcd. 706.4808; found 706.4818 (HR EIMS).

Mutadione D (4d): < 1 mg of a brownish oil, which could only be isolated in admixture with **4a** and **4c**. − TLC: R_f = 0.53, blue spot with ANIS. − UV (CH_3CN): λ_{max} ($abs._{rel}$) = 219 (100), 285 (71) nm. − $C_{42}H_{66}O_7$: calcd. 682.4809; found 682.4767 (HR EIMS).

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