Novel Polyene Carboxylic Acids from *Streptomyces*†

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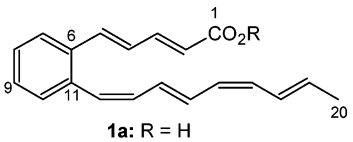
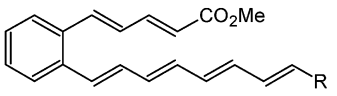
Reinvestigation of the production of the unusual polyene carboxylic acid serpentene (**1a**) from *Streptomyces* sp. Tü 3851 revealed the presence of additional polyene carboxylic acids. The methyl esters of the new all-*trans* serpentene (**2**) and four new dicarboxylic acids (**3**−**6**) were isolated after methylation of the isolated polyene fraction. The dicarboxylic acids might result from ω- and β-oxidation of the parent compounds **1** and **2**.

Serpentene (**1a**) is a unique polyunsaturated carboxylic acid with an *ortho*-substituted benzene ring that is produced exclusively by *Streptomyces* sp. Tü 3851 and shows weak antibacterial activity. In a previous paper about this interesting compound, 60 mg/L of **1a** had been isolated and additional serpentene-like compounds were detected but not isolated.[1] As a prerequisite to understand the biosynthesis of **1a**, we have reinvestigated the production of polyenes in *Streptomyces* sp. Tü 3851. Instead of high amounts of **1a** and similar minor compounds, several polyenes were detected in amounts ranging between 0.4 and 0.8 mg/L upon cultivation of the type strain from the strain collection. Due to the already described sensitivity of **1a** toward light and heat, the cultivation and isolation were performed almost completely in the dark. To furthermore facilitate the separation of the complex polyene mixture, the yellow fraction obtained by gel permeation chromatography (Sephadex LH-20) from the crude extract was methylated with diazomethane, and compounds **1b** and **2**−**6** were isolated by sequential RP-HPLC. Without methylation especially compounds **1b** and **2** and **5** and **6** were almost inseparable.

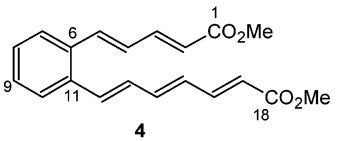
The molecular formulas and structures of these compounds were deduced from HREIMS and detailed 1D (^{1}H and ^{13}C) and 2D (COSY, HMQC, and HMBC) NMR spectral data, respectively. Additional NMR experiments of **1b** obtained after feeding of [1,2-$^{13}C_2$]acetate allowed the correct assignment of some highly similar carbons and overlapping protons (data not shown). All compounds show three spin systems in the COSY experiments: an *ortho*-substituted benzene ring, a pentadiene carboxylic acid methyl ester, and a second polyene chain that was different in all compounds. The configuration of the pentadiene moiety was 2-*E*, 4-*E* in all compounds, as was determined from the coupling constants of $^3J_{H,H} = 15$ Hz for both double bonds. The configurations of the double bonds of the second polyene chain were also determined from the $^3J_{H,H}$ coupling constants. Additionally, characteristic chemical shifts were observed for specific double-bond configurations: a 12-*E* (**2**−**4**) or 12-*Z* (**1b**, **5**, **6**) configuration resulted in a chemical shift of δ_H 7.5−7.6 or 7.2−7.35 for H-10, respectively. The presence of a second methyl ester moiety in **3-6** was readily determined by a second signal in the proton NMR between



1a: R = H
1b: R = Me



2: R = Me
3: R = CO_2Me



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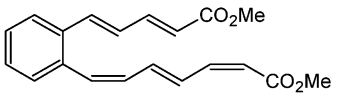

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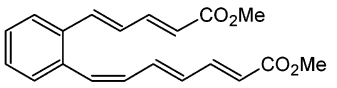

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δ_H 3.7 and 3.8 and in the ^{13}C NMR spectrum between δ_C 51.2 and 51.8, respectively.

A HREI mass spectrum of **2** ($m/z = 306.1620$) implied a molecular formula of $C_{21}H_{22}O_2$, which is identical to the molecular formula of **1b**, therefore indicating the presence of a double-bond isomer. This was confirmed by differences in the ^{1}H NMR spectrum that showed only *E* double bonds ($^3J_{H,H}$ = 15 Hz for **2**). Additionally, a ^{1}H−^{1}H COSY experiment showed the expected three spin systems and allowed the structural assignment. Only 19 carbon signals were detected in the ^{13}C NMR spectrum of **2**, indicating either overlapping or missing carbon atoms. Due to compound decomposition, no further NMR assignments have been obtained, but assignments of characteristic carbon atoms were performed in comparison to compound **1b**.

Compound **3** had an increased mass of 44 amu compared to **2** and showed the presence of two methoxy groups in the ^{1}H (δ_H 3.72 and 3.75) and ^{13}C NMR spectrum (δ_C 51.7 and 51.8), a second ester carbon at δ_C 167.5, and no methyl group. The configurations of all double bonds were found to be *E*; therefore **3** is the C-20 methyl ester derivative of **2**.

† Most of this work was carried out at the Institut für Organische Chemie, Biomolekulare Chemie, Georg-August-Universität Göttingen, Tammannstr. 2, D-37077 Göttingen, Germany.

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Compounds **4**−**6** differ from **3** by 26 amu, indicating the loss of two methine carbons. This is in agreement with NMR data that show signals for 20 protons and carbons, respectively. The configuration of the double bonds was assigned as described before. Additionally, the chemical shifts for H-15 and H-16 in **4**−**6** were strongly influenced by the configuration of the C-16/C-17 double bond. A 16-*E* configuration (**4** and **6**) resulted in chemical shifts of δ_H ~6.5 (H-15) and ~7.4 (H-16), whereas a 16-*Z* configuration (**5**) resulted in the opposite effect with δ_H 7.64 and 6.52 for H-15 and H-16, respectively.

Serpentene (**1a**) and the new derivatives **2**−**6** belong to a rare class of natural products bearing an *ortho*-substituted benzene ring as the central part of an unsaturated carboxylic acid. Demetric acid was isolated from *Streptomyces umbrosus* var. *suragaoensis* and is a C_{18} all-*E* derivative of **1a**.[2] Rubrenoic acids A−C have only 16 carbons: a benzene ring with a hexane or hexene carboxylic acid and a butenyl or butadienyl side chain.[3] They were isolated from the marine bacterium *Alteromonas rubra* and show bronchodilator activity. Even shorter analogues have been described as acyl groups of the *Streptomyces* peptides WS 9326A (14 carbons)[4,5] or RP 1776 (12 carbons).[6] However, so far no dicarboxylic acid derivative has been described. Compounds **3**−**6** are derived most likely from ω-oxidation and double-bond isomerization of serpentene **1a** followed by the well-known fatty acid β-oxidation, which was shown to be highly active in strain Tü 3851 (data not shown). Biochemically all described compounds are very likely derived from fatty acids, polyketides, or hybrids of both, as was confirmed by feeding studies of **1a** with ^{13}C-labeled acetate, which gave the expected labeling pattern (data not shown). To distinguish between these possibilities and especially to elucidate the benzene ring formation, cloning of the serpentene biosynthesis gene cluster is in progress in our group.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 1600 FT-IR instrument and UV spectra on a Varian Cary 3E instrument. EIMS were obtained on a Finnegan MAT 95 (70 eV, direct insert, high resolution with perfluorokerosine as standard), and 1D and 2D NMR experiments were measured on Varian Inova-600 or Varian Inova-500 instruments.

Fermentation. *Streptomyces* sp. Tü 3851 was described previously.[1] For isolation of **1b**−**6** strain Tü 3851 was cultivated for 96 h at 28 °C in 60 × 50 mL of production medium [2% soybean meal (high fat content), 2% mannitol, pH 7.0] in 300 mL Erlenmeyer flasks with three intrusions. The cultures were inoculated with 1 mL of a culture in the same medium grown for 2 days and inoculated with a 1 cm^2 piece of a well-grown agar plate of strain Tü 3851 (production medium, grown for 7 days). All fermentation steps were performed in the dark.

Isolation. All isolation steps were performed in the dark whenever possible. After separation of mycelium and culture filtrate by centrifugation, the culture filtrate was adjusted to pH 6.0 with 0.5 N HCl, defatted with petroleum ether, and adsorbed on Amberlite XAD-2. Elution with methanol resulted in the crude product, which was fractionated on Sephadex LH-20 (methanol). The yellow fraction was evaporated to dryness and dissolved in a small amount of acetone, and diazomethane/ether was added dropwise until the yellow compounds were completely methylated as observed by TLC. After evaporation of the solvent the resulting mixture of esters was dissolved in acetonitrile and separated by HPLC (Jasco Kromasil-100 C18, 0.8 × 25 cm; solvent A: water, solvent B: acetonitrile/water, 99:1; gradient: 80% B for 20 min to 100% B in 10 min; detection at 320 and 366 nm; flow: 2.5 mL/min). The retention times for **1b**−**6** under these conditions were 23.4, 25.0, 13.4, 10.0, 8.4, and 9.1 min, respectively, and 2.5, 1.0, 1.8, 2.1, 2.4, and 1.4 mg of **1b**−**6**, respectively, were isolated.

Serpentene methyl ester (1b): yellow amorphous solid; UV (MeOH) λ_{max} (log ϵ) 298 (4.14) nm; IR (KBr) ν_{max} 2929, 2832, 1703, 1682, 1596, 1382, 1365, 1268, 1137, 776 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.83 (d, 3H, J = 7.0 Hz, H_3-20), 3.74 (s, 3H, 1-OCH_3), 5.76 (dq, 1H, J = 7.0, 15.0 Hz, H-19), 5.83 (dd, 1H, J = 11.0, 11.0 Hz, H-16), 5.96 (dd, 1H, J = 11.0, 11.0 Hz, H-17), 5.96 (d, 1H, J = 15.0 Hz, H-2), 6.38 (dd, 1H, J = 11.0, 14.0 Hz, H-14), 6.44 (dd, 1H, J = 11.0, 11.0 Hz, H-13), 6.51 (d, 1H, J = 10.5 Hz, H-12), 6.56 (m, 1H, H-18), 6.79 (dd, 1H, J = 11.0, 15.0 Hz, H-4), 6.80 (dd, 1H, J = 11.0, 14.0 Hz, H-15), 7.07 (d, 1H, J = 15.5 Hz, H-5), 7.24−7.29 (m, 3H, H-8, H-9, H-10), 7.42 (dd, 1H, J = 11.0, 15.0 Hz, H-3), 7.57 (m, 1H, H-7); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 18.5 (CH_3, C-20), 51.6 (CH_3, 1-OCH_3), 120.8 (CH, C-2), 125.9 (CH, C-7), 126.9 (CH, C-16), 127.1 (CH, C-18), 127.5 (CH, C-4; CH, C-10), 128.0 (CH, C-12), 128.5 (CH, C-9), 128.7 (CH, C-14), 130.6 (CH, C-8), 130.9 (CH, C-15; CH, C-17), 131.7 (CH, C-19), 132.1 (CH, C-13), 134.5 (C, C-6), 136.9 (C, C-11), 138.6 (CH, C-5), 145.1 (CH, C-3), 167.5 (C, C-1); EIMS m/z 306 [M$^+$] (48), 191 (32), 178 (64), 165 (100), 153 (38), 141 (47), 128 (46), 119 (30), 91 (34), 59 (30); HREIMS m/z 306.1620 (calcd for $C_{21}H_{22}O_2$, 306.1620).

Compound 2: yellow amorphous solid; UV (MeOH) λ_{max} (log ϵ) 297 (3.42) nm; IR (KBr) ν_{max} 2924, 1704, 1631, 1543, 1382, 1264, 1058, 564 cm^{-1}; ^{1}H NMR (500 MHz, CD_2Cl_2) δ 1.85 (d, 3H, J = 7.0 Hz, H_3-20), 3.78 (s, 3H, 1-OCH_3), 5.80 (dq, 1H, J = 7.0, 15.0 Hz, H-19), 6.01 (d, 1H, J = 15.0 Hz, H-2), 6.16 (ddd, 1H, J = 1.5, 10.0, 15.0 Hz, H-18), 6.23 (dd, 1H, J = 9.5, 15.0 Hz, H-16), 6.30 (dd, 1H, J = 10.0, 14.5 Hz, H-17), 6.41 (dd, 1H, J = 10.0, 14.0 Hz, 1H, H-15), 6.43 (dd, 1H, J = 10.5, 14.0 Hz, H-14), 6.76 (dd, 1H, J = 10.0, 15.0 Hz, H-13), 6.81 (dd, 1H, J = 11.0, 15.0 Hz, H-4), 6.86 (d, 1H, J = 15.0 Hz, H-12), 7.21−7.31 (m, 3H, H-5, H-8, H-9), 7.48 (ddd, 1H, J = 1.0, 11.0, 15.0 Hz, H-3), 7.50−7.54 (m, 2H, H-7, H-10); ^{13}C NMR (125.7 MHz, CD_2Cl_2) δ 18.6 (CH_3, C-20), 51.8 (CH_3, 1-OCH_3), 121.4 (CH, C-2), 126.5 (CH), 126.9 (CH), 127.8 (CH), 128.5 (CH), 128.7 (CH), 129.3 (CH), 130.7 (CH), 131.4 (CH), 132.2 (CH), 132.6 (CH), 132.7 (CH), 134.7 (CH), 135.0 (CH), 138.3 (CH, C-5), 145.1 (CH, C-3), 167.5 (C, C-1); EIMS m/z 306 [M$^+$] (100), 191 (25), 179 (40), 165 (72), 153 (32), 128 (26); HREIMS m/z 306.1621 (calcd for $C_{21}H_{22}O_2$, 306.1620).

Compound 3: yellow amorphous solid; UV (MeOH) λ_{max} (log ϵ) 378 (4.34), 317 (4.19) nm; IR (KBr) ν_{max} 2927, 1752, 1686, 1650, 1403, 1320, 1199, 1096, 1066, 992, 964, 911, 774, 592 cm^{-1}; ^{1}H NMR (500 MHz, CD_2Cl_2) δ 3.72/3.75 (s, 3H, H_3-1 and H_3-20), 5.91 (d, 1H, J = 15.0 Hz, H-19), 6.03 (d, 1H, J = 15.0 Hz, H-2), 6.43 (dd, 1H, J = 11.5, 15.0 Hz, H-17), 6.49 (dd, 1H, J = 11.0, 15.0 Hz, H-15), 6.65 (dd, 1H, J = 11.0, 15.0, H-14), 6.70 (dd, 1H, J = 11.0, 15.0 Hz, H-16), 6.81 (dd, 1H, J = 11.0, 15.0 Hz, H-13), 6.82 (dd, 1H, J = 11.5, 15.0 Hz, H-4), 7.00 (d, 1H, J = 15.0 Hz, H-5), 7.28 (d, 1H, J = 15.0 Hz, H-12), 7.25−7.33 (m, 2H, H-8, H-9), 7.34 (dd, 1H, J = 11.5, 15.5 Hz, H-18), 7.48 (dd, 1H, J = 11.0, 15.5 Hz, H-3), 7.53−7.56 (m, 2H, H-7, H-10); ^{13}C NMR (125.7 MHz, CD_2Cl_2) δ 51.7/51.8 (CH_3, 1-OCH_3/20-OCH_3), 120.9 (CH, C-19), 121.6 (CH, C-2), 126.7 (CH, C-7 or C-10), 127.0 (CH, C-7 or C-10), 128.4 (CH, C-8 or C-9), 128.9 (CH, C-13), 129.3 (CH, C-8 or C-9), 130.9 (CH, C-17), 131.8 (CH, C-12), 131.9 (CH, C-4), 133.3 (CH, C-15), 134.8 (C, C-6), 136.4 (C, C-11), 137.5 (CH, C-14), 138.0 (CH, C-5), 140.9 (CH, C-16), 144.6 (CH, C-18), 144.9 (CH, C-3), 167.5 (C, C-1/C-20); EIMS m/z 350 [M$^+$] (100), 231 (24), 191 (32), 165 (40), 128 (26); HREIMS m/z 350.1520 (calcd for $C_{22}H_{22}O_4$, 350.1518).

Compound 4: yellow amorphous solid; UV (MeOH) λ_{max} (log ϵ) 347 (4.11), 307 (4.18) nm; IR (KBr) ν_{max} 3024, 2949, 1712, 1623, 1435, 1303, 1261, 1242, 1174, 1136, 1002, 756 cm^{-1}; ^{1}H NMR (500 MHz, CD_2Cl_2) δ 3.73/3.75 (s, 3H, H_3-1 and H_3-18), 5.95 (d, 1H, J = 15.5 Hz, H-17), 6.03 (d, 1H, J = 15.0 Hz, H-2), 6.51 (dd, 1H, J = 11.0, 14.0 Hz, H-15), 6.79 (dd, 1H, J = 11.0, 14.0 Hz, H-14), 6.82 (dd, 1H, J = 10.5, 14.0 Hz, H-13), 6.83 (ddd, 1H, J = 1.0, 11.0, 15.5 Hz, H-4), 7.08 (d, 1H, J = 14.0 Hz, H-12), 7.28 (d, 1H, J = 15.5 Hz, H-5), 7.27−7.33 (m, 2H, H-8, H-9), 7.38 (dd, 1H, J = 11.5, 15.5 Hz, H-16), 7.49 (ddd, 1H, J = 1.0, 11.0, 14.5 Hz, H-3), 7.54−7.57 (m, 2H, H-7, H-10);

^{13}C NMR (125.7 MHz, CD_2Cl_2) δ 51.8 (CH_3, 1-O*C*H$_3$/20-O*C*H$_3$), 121.4 (CH, C-17), 121.7 (CH, C-2), 126.9 (CH, C-7 or C-10), 127.0 (CH, C-7 or C-10), 128.8 (CH, C-8 or C-9), 129.1 (CH, C-4), 129.3 (CH, C-8 or C-9), 131.3 (CH, C-13, C-15), 133.6 (CH, C-12), 135.0 (C, C-6), 136.0 (C, C-11), 137.8 (CH, C-5), 141.0 (CH, C-14), 144.5 (CH, C-16), 144.9 (CH, C-3), 167.5 (C, C-1/C-18); EIMS *m*/*z* 324 [M$^+$] (100), 232 (42), 205 (66), 178 (34), 165 (100), 128 (36), 111 (18); HREIMS *m*/*z* 324.1359 (calcd for $C_{20}H_{20}O_4$, 324.1361).

Compound 5: yellow amorphous solid; UV (MeOH) λ_{max} (log ϵ) 356 (4.33), 311 (4.14) nm; IR (KBr) ν_{max} 2926, 2854, 1720, 1623, 1458, 1437, 1267, 1170, 1136, 1075, 1004, 756 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.72/3.73 (s, 3H, H$_3$-1 and H$_3$-20), 5.64 (d, 1H, J = 11.0 Hz, H-17), 5.97 (d, 1H, J = 15.0 Hz, H-2), 6.52 (dd, 1H, J = 11.5, 11.5 Hz, H-16), 6.53 (dd, 1H, J = 11.5, 11.5 Hz, H-13), 6.65 (ddd, 1H, J = 1.0, 11.5, 15.0 Hz, H-14), 6.74 (d, 1H, J = 11.0 Hz, H-12), 6.79 (dd, 1H, J = 11.0, 15.5 Hz, H-4), 7.04 (d, 1H, J = 15.5 Hz, H-5), 7.22−7.24 (m, 1H, H-10), 7.29 (m, 2H, H-8, H-9), 7.41 (ddd, 1H, J = 1.0, 11.0, 15.0 Hz, H-3), 7.59 (m, 1H, H-7), 7.64 (dd, 1H, J = 11.5, 15.0 Hz, H-15); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 51.2/51.6 (CH_3, 1-O*C*H$_3$/20-O*C*H$_3$), 117.5 (CH, C-17), 121.2 (CH, C-2), 126.1 (CH, C-7), 127.9 (CH, C-4 or C-8), 128.0 (CH, C-4 or C-8), 128.5 (CH, C-9), 130.5 (CH, C-10), 131.0 (CH, C-15), 131.4 (CH, C-13), 132.4 (CH, C-12), 134.7 (C, C-6), 136.2 (C, C-11), 137.2 (CH, C-14), 138.2 (CH, C-5), 144.4 (CH, C-16), 144.8 (CH, C-3), 166.8 (C, C-18), 167.4 (C, C-1); EIMS *m*/*z* 324 [M$^+$] (56), 292 (44), 260 (24), 232 (52), 205 (70), 178 (38), 165 (100), 128 (44), 111 (36); HREIMS *m*/*z* 324.1360 (calcd for $C_{20}H_{20}O_4$ 324.1361).

Compound 6: yellow amorphous solid; UV (MeOH) λ_{max} (log ϵ) 350 (4.12), 306 (4.26) nm; IR (KBr) ν_{max} 2923, 2853, 1736, 1719, 1631, 1542, 1382, 1267, 1170, 1007, 759 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.70/3.75 (s, 3H, H$_3$-1 and H$_3$-20), 5.89 (d, 1H, J = 15.5 Hz, H-17), 5.97 (d, 1H, J = 15.5 Hz, H-2), 6.44 (dd, 1H, J = 11.0, 14.5 Hz, H-15), 6.44 (dd, 1H, J = 11.5, 11.5 Hz, H-13), 6.72 (dd, 1H, J = 11.5, 14.5 Hz, H-14), 6.74 (d, 1H, J = 11.0 Hz, H-12), 6.80 (dd, 1H, J = 11.0, 15.5 Hz, H-4), 7.02 (d, 1H, J = 15.5 Hz, H-5), 7.23 (m, 2H, H-10, H-16), 7.25−7.35 (m, 2H, H-8, H-9), 7.40 (ddd, 1H, J = 11.0, 15.0 Hz, H-3), 7.59 (m, 1H, H-7); ^{13}C NMR (150.0 MHz, $CDCl_3$) δ 51.6 (CH_3, 1-O*C*H$_3$/20-O*C*H$_3$), 121.2 (CH, C-2, C-17), 126.1 (CH, C-7), 127.9 (CH, C-4), 128.1 (CH, C-8 or C-9), 128.6 (CH, C-8 or C-9), 130.5 (CH, C-10), 130.8 (CH, C-13 or C-15), 132.2 (CH, C-13 or C-15), 132.6 (CH, C-12), 134.6 (C, C-6), 136.0 (C, C-11), 136.6 (CH, C-14), 138.1 (CH, C-5), 144.4 (CH, C-16), 144.8 (CH, C-3), 167.3 (C, C-1 or C-18), 167.4 (C, C-1 or C-18); EIMS *m*/*z* 324 [M$^+$] (44), 233 (28), 205 (50), 179 (65), 165 (100), 141 (38), 128 (46), 111 (23); HREIMS *m*/*z* 324.1363 (calcd for $C_{20}H_{20}O_4$, 324.1361).

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