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**Total synthesis and biological evaluation of (-)-pectinatone employing a
methyl-branced was ester as key building block
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**Total Synthesis and Biological Evaluation of (–)-Pectinatone Employing a Methyl
Branched Wax Ester as Key Building Block**

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Unnatural (–)-pectinatone (–)-**3** was prepared in five steps starting from the highly methyl-branched wax ester **4** employing bromination of the ester enolate and subsequent base-induced elimination to the enoate **6** as the key step. Both (–)-pectinatone **3** and the amides **8b,c**, which were isolated as by-products in the reaction sequence, displayed antimicrobial activity and cytotoxicity.

Introduction. – Gastropod mollusks of the genus *Siphonaria* which are living in the intertidal region of the Mediterranean sea and Atlantic ocean produce several antimicrobially active polypropionates such as (+)-siphonarinal (**1**) [1], (+)-siphonarinenone (**2**) [2], and (+)-pectinatone (**3**) [2,3] (Scheme 1) as part of their chemical defense strategy [4].

Scheme 1

(+)-Pectinatone (+)-**3**, first isolated by Ireland [3b] from the skin extract of the pulmonate *Siphonaria pectinata* collected on the sea wall at the entrance of Key Biscayne (Florida), displayed antibiotic properties against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* as well as yeasts *Candida albicans* and *Saccharomyces cerevisiae*. The originally proposed configuration of (+)-**3** was later revised by Garson [3a] as shown in Scheme 1.

As a part of our ongoing program on polypropionate-containing natural products and their unnatural enantiomers, we recently reported the "ex chiral pool" synthesis of (–)-siphonarinenone (–)-**2** starting from the methyl-branched wax ester **4** [5,6] (Scheme 2) which is obtained from preen-gland wax of the domestic goose *Anser a. f. domesticus* [7]. Herein

the results towards the total synthesis of (–)-pectinatone (–)-**3** and its biological evaluation are discussed.

Scheme 2

Results and Discussion. – As shown in Scheme 3, we first reinvestigated the critical α -hydroxylation/elimination step from wax ester **4** to the unsaturated precursor **6** which was accompanied by the competing formation of regioisomeric enoate **7**. Following a procedure by Ando [8], methyl (2*R*,4*R*,6*R*,8*R*)-2,4,6,8-tetramethylundecanoate (**4**) was deprotonated with LDA and then quenched with CBr_4 to give compounds **5** (dr 66 : 34) in 87% yield. The latter were simply heated in pyridine, giving the target ester **6** as the major product in 59% yield.

Scheme 3

In contrast, both the reaction with phenylselenenyl bromide via the α -phenylselenenyl ester [5,9] followed by selenoxide elimination in the presence of MCPBA [10] and the reaction via the diastereomeric α -hydroxyesters [5,11], direct tosylation in the presence of 2,6-di-*tert*-butyl-4-methylpyridine [12] and subsequent heating with DBU in toluene at 135°C [13] gave lower yields of **6**.

Having accomplished this elimination, the further synthesis route to the unnatural pectinatone (–)-**3** followed our previously described protocol [5] (Scheme 4).

Scheme 4

The α,β -unsaturated ester **6** was converted into the Weinreb amide **8a** in 81% yield by treatment with Me(OMe)NH · HCl, addition of *i*PrMgCl and final hydrolysis with NH₄Cl. In the next step, ethyl 2-methyl-3-oxopentanoate **9**, which is accessible by Claisen condensation of ethyl propionate in the presence of NaH [14], was deprotonated with LDA in THF at 0°C and then reacted with the Weinreb amide **8a** to yield a mixture of the β,δ -diketo ester **10** (22%) and the amides **8b** (26%) and **8c** (40%). The formation of amide **8c**

from precursor **8a** was also observed by Enders in the synthesis of the natural (+)-pectinatone (+)-**3** [6f]. Final cyclization of β,δ -diketo ester **10** with DBU in toluene at 130°C proceeded without any problem and the desired (-)-pectinatone (-)-**3** was isolated in 88% yield.

Scheme 5

(-)-Pectinatone (-)-**3** and both amides **8b,c** were submitted to an antimicrobial agar diffusion assay (20 μ g/6-mm paper disc) employing Gram-negative (*E. coli* tolC, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus*, *Mycobacterium phlei*), yeasts (*Candida albicans*, *Hansenula anomala*, *Saccharomyces cerevisiae*) as well as hyphal fungi (*Aspergillus fumigatus*, *Botrytis cinerea*, *Pythium debaryanum*) and a cell proliferation assay with L929 mouse fibroblasts (Table 1). Additionally, the data of both diastereomeric allylic alcohols **11a** and **11a'** (Scheme 5) and pure siphonarienones (+)-**2** and (-)-**2** are listed.

Table 1

As can be seen from Table 1, among the derivatives investigated amides **8** and pectinatone (-)-**3** exhibited the highest cytotoxicity. While the cytotoxicity of **8b** against L929 mouse fibroblasts resembles that of **8c**, the cytotoxic activity of (-)-pectinatone (-)-**3** was only slightly diminished to 15 μ g/ml. Unfortunately, a direct comparison with enantiomer (+)-**3** is not possible and moreover, detailed information of biological tests are missing in literature. The IC₅₀ values for both diastereomeric mixture of allylic alcohols **11a** and **11a'**

were identical. However, enantiopure siphonarienone (+)-**2** and (–)-**2** significantly differ with 25 µg/ml and 40 µg/ml [5], respectively (Table 1).

In this series of compounds only the unnatural pectinatone (–)-**3** and amide **8c** displayed any antimicrobial activity under the used conditions. As reported for natural (+)-**3** [3], the congener (–)-**3** was active against Gram-positive bacteria *Staphylococcus aureus* and *Micrococcus luteus* (zone of inhibition 13 mm and 11 mm, respectively), while amide **8c** weakly inhibited the growth of the sensitive mutant *E. coli* tolC (zone of inhibition 7 mm) (Table 1).

Upon comparison of these results, it seems that the polypropionate skeleton induces cytotoxicity whereas the antibiotic activity is related to the α -pyrone moiety. This structure-activity consideration is in good agreement with results by Garson [3a], Norte [6a], and Faulkner [15].

Conclusions. – A five-step "ex chiral pool" synthesis of (–)-pectinatone (–)-**3** starting from the enantiopure methyl-branched wax ester **4** has been realized. Electrophilic bromination of the ester enolate from **4** and subsequent base-catalyzed elimination provided the α,β -unsaturated ester **6** as a key intermediate. Based on the biological assays, the antibiotic properties of (–)-pectinatone (–)-**3** are assumed to be associated with the α -pyrone moiety while the unnatural polypropionate chain seems to induce cytotoxicity.

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Experimental Part

General. FT-IR Spectra: $\tilde{\nu}$ in cm^{-1} . NMR Spectra: *Bruker AC 250 F* and *Bruker ARX 500*; δ in ppm and J in Hz. MS (FAB): m/z (rel. %). GC: *Hewlett-Packard HP 6890*, HP-5 column ($30\text{ m} \times 0.32\text{ mm}$), H_2 as carrier gas (flow 2 ml min^{-1}); temperature program: 8°C min^{-1} gradient from 80°C to 280°C . All solvent were dried, and reactions were performed in dried glassware.

Methyl (2RS,4R,6R,8R)-2-bromo-2,4,6,8-tetramethylundecanoate (5). To a 1.5 M soln. of *n*-BuLi in hexane ($390\text{ }\mu\text{l}$, 0.59 mmol) at -78°C was added dropwise abs. diisopropylamine ($82\text{ }\mu\text{l}$, 0.59 mmol) followed by addition of abs. THF (2 ml), and the reaction mixture was stirred for 30 min at -78°C . A soln. of **4** (100 mg , 0.39 mmol) in abs. THF (3 ml) was slowly added dropwise and the reaction mixture stirred for 40 min . Then a soln. of CBr_4 (194 mg , 0.59 mmol) in abs. THF (2 ml) was slowly added dropwise, the reaction mixture stirred for a further 40 min , hydrolyzed at -78°C with a soln. of 10% $\text{NH}_4\text{Cl-H}_2\text{O}$ (5 ml) and warmed to r.t.. The aqueous layer was separated and extracted with Et_2O ($3 \times 10\text{ ml}$). The combined organic layers were dried (Na_2SO_4) and the solvent removed under vacuum ($30^\circ\text{C}/10\text{ mbar}$). The residue was purified by chromatography on SiO_2 (hexanes/ EtOAc $50 : 1$, $R_f = 0.32$) to give a diastereomeric mixture (dr $34 : 66$) of **5** (114 mg , 0.34 mmol , 87%) as a yellow oil. GC: $t_R = 13.47\text{ min}$ (minor diastereomer), $t_R = 13.59\text{ min}$ (major diastereomer). FT-IR (ATR): 2953 (s) , 2914 (s) , 2871 (s) , 2843 (s) , 1737 (vs) , 1456 (m) , 1379 (m) , 1222 (m) , 1180 (m) . $^1\text{H-NMR}$ (500 MHz , CDCl_3): $0.80\text{ (d, } J = 6.6, \text{ Me(8))}$, $0.84\text{ (d, } J = 6.6, \text{ Me(6))}$, $0.88\text{ (t, } J = 7.1, \text{ H}_3\text{C(11))}$, $0.92\text{ (d, } J = 6.5, \text{ Me(4))}$, $0.95\text{--}1.05\text{ (m, H}_a\text{-C(7))}$, $1.09\text{--}1.35\text{ (m, H}_a\text{-C(10), H}_b\text{-C(10), H}_a\text{-C(9), H}_b\text{-C(9), H}_b\text{-C(7))}$, $1.36\text{--}1.45\text{ (m, H}_a\text{-C(5), H}_b\text{-C(5))}$, $1.46\text{--}1.55\text{ (m, H-C(6), H-C(8))}$, $1.56\text{--}1.66\text{ (m, H-C(4))}$, $1.85\text{ (d, } J = 9.9, \text{ H}_3\text{C(2))}$, $2.05\text{--}2.20\text{ (m, H}_a\text{-C(3), H}_b\text{-C(3))}$, 3.77 (s, OMe) . $^{13}\text{C-NMR}$ (125 MHz ,

CDCl₃): 19.6 (C(10)), 19.9 (Me(6)), 20.1 (Me(6))* , 20.2 (Me(4)), 20.4 (Me(4))* , 21.1 (Me(8)), 21.2 (Me(8))* , 26.7 (Me(2)), 26.8 (Me(2))* , 27.2 (C(8)), 28.2 (C(6)), 28.5 (C(6))* , 29.2 (C(4)), 29.3 (C(4))* , 38.3 (C(9))* , 38.5 (C(9)), 44.4 (C(7))* , 44.7 (C(7)), 45.7 (C(5))* , 46.0 (C(5)), 48.7 (C(3)), 49.5 (C(3))* , 52.5 (OMe), 52.6 (OMe)* , 60.3 (C(2)), 61.1 (C(2))* , 171.8 (C(1))* , 172.1 (C(1)). (* signals of the minor diastereomer) MS (EI): 334.2 (3), 255.2 (25), 185.1 (15), 168.0 (40), 127.1 (10), 111.1 (20), 101.0 (100), 88.0 (45), 71.1 (45), 57.0 (50), 43.0 (75). HR-MS (EI): calcd. for C₁₆H₃₁BrO₂ 334.1507, found ⁷⁹Br 334.1508 [M⁺], ⁸¹Br 334.1488 [M⁺].

Methyl (2E,4R,6R,8R)-2,4,6,8-tetramethyl-2-undecenoate (6). A soln. of **5** (180 mg, 0.54 mmol) in abs. pyridine (7 ml) was stirred at 120°C for 16 h. After cooling to r.t., CH₂Cl₂ (7 ml) was added and the reaction mixture washed with 1 M HCl (5 ml). The aqueous layer was separated and extracted with Et₂O (3 × 5 ml). The combined organic layers were dried (Na₂SO₄) and the solvent removed under vacuum (35°C/10 mbar). The residue was purified by chromatography on SiO₂ (hexanes/CH₂Cl₂ 4 : 1) to give in a first fraction (R_f = 0.25) enoate **7** (17 mg, 66.8 μmol, 12%) and in a second fraction (R_f = 0.15) product **6** (82 mg, 0.31 mmol, 59%) as colorless oils. The spectroscopic data were in accordance with the literature [5].

Ethyl (2RS,4RS,6E,8R,10R,12R)-2,4,6,8,10,12-hexamethyl-6-pentadecenoate (10). Abs. diisopropylamine (103 μl, 0.73 mmol) was added dropwise to a 1.5 M soln. of *n*-BuLi in hexane (0.5 ml, 0.73 mmol) at 0°C. After addition of abs. THF (1 ml), the reaction mixture was stirred for 15 min at 0°C and then a soln. of **9** (58 mg, 0.37 mmol) in abs. THF (1 ml) was slowly added dropwise. The reaction mixture was stirred for 30 min. A soln. of **8a** [5] (70 mg, 0.24 mmol) in abs. THF (1 ml) was slowly added dropwise, the reaction mixture

stirred for a further 30 min at 0°C, hydrolyzed with 5% NH₄Cl–H₂O (1 ml) and warmed to r.t.. The aqueous layer was separated and extracted with Et₂O (3 × 5 ml). The combined organic layers were dried (Na₂SO₄) and the solvent removed under vacuum (30°C/10 mbar). Chromatographic purification on SiO₂ (hexanes/EtOAc 20 : 1, R_f = 0.31) gave a diastereomeric mixture (dr 31:43:16:10) of **10** (20 mg, 52.6 μmol, 22%) as a colorless oil. A second chromatography on SiO₂ (hexanes/EtOAc 3 : 1) gave in a first fraction (R_f = 0.30) **8b** (18 mg, 63.5 μmol, 26%) and in second fraction (R_f = 0.21) **8c** (25 mg, 98.6 μmol, 40%) as colorless oils. Compound **10**: GC: t_R = 18.82 min (diastereomer 1), t_R = 19.07 min (diastereomer 2), t_R = 19.19 min (diastereomer 3), t_R = 19.29 min (diastereomer 4). FT-IR: 2956 (s), 2927 (s), 2871 (s), 2844 (s), 1747 (vs), 1718 (vs), 1659 (vs), 1454 (s), 1375 (s), 1191(m), 1037 (m). ¹H-NMR (500 MHz, CDCl₃): 0.79–0.88 (*m*, Me(21), Me(20), H–C(15)), 0.90–0.98 (*m*, H_a–C(11)), 1.00–1.05 (*m*, Me(19)), 1.06–1.19 (*m*, H_b–C(11), H_a–C(14)), 1.20–1.38 (*m*, Me(17), Me(16), OCH₂CH₃, H_a–C(13), H_a–C(9), H_b–C(14), H_b–C(13), H_b–C(9)), 1.39–1.52 (*m*, H–C(10), H–C(12)), 1.82 (*s*, Me(18)), 2.66–2.78 (*m*, H–C(8)), 3.57 (*dq*, *J* = 6.9, *J* = 9.5, H–C(4)), 3.68 (*dq*, *J* = 3.1, *J* = 7.1, H–C(4))* , 4.07–4.22 (*m*, OCH₂CH₃), 4.42–4.48 (*m*, H–C(2))* , 4.59 (*q*, *J* = 7.0, H–C(2)), 6.38–6.45 (*m*, H–C(7)). ¹³C-NMR (125 MHz, CDCl₃): 11.6 (Me(18)), 11.7 (Me(18))* , 12.8 (Me(17)), 12.9 (Me(17))* , 13.6 (Me(16)), 13.7 (Me(16))* , 14.1 (Me(16))* , 14.2 (OCH₂CH₃), 14.3 (OCH₂CH₃)* , 14.4 (C(15)), 19.9 (C(14)), 20.1 (Me(20)), 20.2 (Me(20))* , 20.3 (Me(21)), 20.4 (Me(21))* , 20.5 (Me(19)), 20.6 (Me(19))* , 28.2 (C(10)), 28.3 (C(10))* , 28.4 (C(10))* , 29.6 (C(12)), 31.5 (C(8))* , 31.6 (C(8))* , 31.7 (C(8)), 39.0 (C(9))* , 39.1 (C(9))* , 39.2 (C(9)), 39.3 (C(9))* , 44.3 (C(13))* , 44.4 (C(13))* , 44.5 (C(13)), 45.5 (C(11)), 45.6 (C(11))* , 45.7 (C(11))* , 50.0 (C(2))* , 50.1 (C(2))* , 51.1 (C(2))* , 51.2 (C(2)), 53.1 (C(4)), 53.2 (C(4))* , 53.6 (C(4))* , 53.7 (C(4))* , 61.3 (OCH₂CH₃)* , 61.4 (OCH₂CH₃), 134.6 (C(6)), 134.7 (C(6))* , 134.9 (C(6))* , 135.0 (C(6))* , 150.8 (C(7))* , 151.0 (C(7))* , 151.1

(C(7))* , 151.2 (C(7)), 170.1 (C(1))* , 170.2 (C(1))* , 170.3 (C(1))* , 170.4 (C(1)), 198.2 (C(5))* , 198.3 (C(5))* , 199.6 (C(5)), 199.7 (C(5))* , 202.2 (C(3)), 202.3 (C(3))* , 203.3 (C(3))* , 203.4 (C(3))* . (* signals of the minor diastereomer) MS (EI): 380.3 (45), 335.3 (15), 279.2 (10), 225.1 (30), 223.2 (100), 181.1 (15), 158.1 (10), 125.1 (15), 111.1 (30), 109.1 (65), 69.1 (35), 43.0 (30). HR-MS (EI): calcd. for C₂₃H₄₀O₄ 380.2927, found 380.2925 [M⁺]. Amide **8b**: $[\alpha]_{\text{D}}^{20} = -24.4$ (*c* = 0.5, CDCl₃). GC: *t*_R = 15.59 min. FT-IR: 3341 (b), 2955 (s), 2925 (s), 2870 (s), 2843 (s), 1742 (m), 1713 (m), 1663 (s), 1626 (s), 1519 (s), 1455 (s), 1310 (m), 1070 (m). ¹H-NMR (500 MHz, CDCl₃): 0.80 (*d*, *J* = 6.5, Me(8)), 0.82 (*d*, *J* = 6.5, Me(6)), 0.87 (*t*, *J* = 7.1, H-C(11)), 0.88–0.97 (*m*, H_a-C(7)), 0.98 (*d*, *J* = 6.7, Me(4)), 1.00–1.10 (*m*, H_a-C(9), H_a-C(10)), 1.12–1.18 (*m*, H_a-C(5), H_b-C(7)), 1.20–1.28 (*m*, H_b-C(10), H_b-C(9)), 1.29–1.35 (*m*, H_b-C(5)), 1.28–1.52 (*m*, H-C(8), H-C(6)), 1.89 (*s*, Me(2)), 2.51–2.62 (*m*, H-C(4)), 3.35 (*s*, NMe), 4.76 (*d*, *J* = 6.9, NCH₂), 6.12 (*d*, *J* = 9.6, H-C(3)), 6.32 (*bs*, OH). ¹³C-NMR (125 MHz, CDCl₃): 12.8 (Me(2)), 14.4 (C(11)), 19.9 (C(10)), 20.1 (Me(6)), 20.4 (Me(8)), 20.8 (Me(4)), 28.1 (C(8)), 29.6 (C(6)), 30.6 (C(4)), 39.3 (C(9)), 44.5 (C(7)), 45.6 (C(5)), 56.0 (NMe), 71.7 (NCH₂), 129.1 (C(2)), 143.2 (C(3)), 169.9 (C(1)). MS (CI): 284.3 (100), 269.3 (25), 251.2 (25), 240.2 (30), 223.2 (35), 180.2 (10), 151.1 (10), 128.1 (15), 109.1 (25), 96.1 (13), 86.1 (10), 69.1 (10), 45.0 (10). HR-MS (CI): calcd. for C₁₇H₃₃NO₂ 284.2589 [MH⁺], found 284.2594. Amide **8c**: $[\alpha]_{\text{D}}^{20} = -25.2$ (*c* = 0.5, CDCl₃). GC: *t*_R = 14.32 min. FT-IR: 3339 (b), 2955 (s), 2926 (s), 2871 (s), 2843 (s), 1742 (m), 1714 (m), 1661 (m), 1616 (s), 1533 (s), 1455 (s), 1377 (m). ¹H-NMR (300 MHz, CDCl₃): δ = 0.79 (*d*, *J* = 6.6, Me(6)), 0.81 (*d*, *J* = 6.5, Me(8)), 0.87 (*t*, *J* = 7.1, H-C(11)), 0.96 (*d*, *J* = 6.6, Me(4)), 0.98–1.53 (*m*, H_a-C(10), H_b-C(10), H_a-C(9), H_b-C(9), H_a-C(7), H_b-C(7), H_a-C(5), H_b-C(5), H-C(8), H-C(6)), 1.86 (*s*, Me(2)), 2.50–2.65 (*m*, H-C(4)), 2.86 (*d*, *J* = 4.8, NMe), 5.71 (*bs*, H-N), 6.04 (*d*, *J* = 9.9, H-C(3)). ¹³C-NMR (125 MHz, CDCl₃): 12.9 (Me(2)), 14.4 (C(11)), 19.9 (C(10)), 20.1 (Me(6)), 20.4

(Me(8)), 20.9 (Me(4)), 26.5 (C(8)), 28.1 (C(6)), 29.6 (C(4)), 30.5 (NMe), 39.2 (C(9)), 44.5 (C(7)), 45.6 (C(5)), 129.3 (C(2)), 141.9 (C(3)), 170.2 (C(1)). MS (EI): 253.2 (12), 238.2 (2), 223.1 (1), 182.1 (12), 140.1 (50), 100.0 (100), 87.0 (100), 69.0 (20), 58.1 (15), 43.0 (30). HR-MS (EI): calcd. for $C_{16}H_{31}NO$ 253.2406, found 253.2405 [M^+].

Pectinatone (**3**). Under N_2 atmosphere DBU (0.6 μ l) was added to a soln. of **10** (18 mg, 47.3 μ mol) in abs. toluene (1.5 ml), the reaction mixture stirred for 3.5 h at 130°C and then cooled to r.t.. After addition of CH_2Cl_2 (5 ml), the reaction mixture was washed with 1 M HCl. The layers were separated and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were dried (Na_2SO_4) and the solvent removed under vacuum (35°C/10 mbar). The residue was purified by chromatography on SiO_2 (hexanes/ $EtOAc$ 2 : 1, R_f = 0.22) to give (–)-**3** (14 mg, 41.8 μ mol, 88%) as a colorless oil, $[\alpha]_D^{20} = -65.0$ (c = 0.12, $CDCl_3$). GC: t_R = 21.49 min. FT-IR: 3220 (b), 2955 (s), 2924 (s), 2869 (s), 2844 (s), 1656 (vs), 1559 (vs), 1455 (s), 1378 (s), 1225 (s), 1080 (m). 1H -NMR (500 MHz, $CDCl_3$): 0.81 (d, J = 6.5, Me(13)), 0.86 (d, J = 6.5, Me(11)), 0.87 (t, J = 7.0, H–C(16)), 0.90–0.99 (m, H_a –C(12)), 0.99 (d, J = 6.6, Me(9)), 1.00–1.10 (m, H_b –C(12), H_a –C(15)), 1.12–1.18 (m, H_a –C(14)), 1.20–1.35 (m, H_a –C(10), H_b –C(15), H_b –C(14), H_b –C(10), H–C(13), OH), 1.42–1.52 (m, H–C(11)), 1.91 (s, Me(7)), 2.00 (s, Me(3)), 2.01 (s, Me(5)), 2.58–2.69 (m, H–C(9)), 5.36 (d, J = 9.6, H–C(8)). ^{13}C -NMR (125 MHz, $CDCl_3$): 8.4 (Me(3)), 11.4 (Me(5)), 14.4 (C(16)), 14.8 (Me(7)), 19.9 (C(15)), 20.1 (Me(11)), 20.2 (Me(13)), 21.2 (Me(9)), 28.3 (C(13)), 29.6 (C(11)), 30.5 (C(9)), 39.4 (C(14)), 44.7 (C(12)), 45.8 (C(10)), 98.6 (C(3)), 105.5 (C(5)), 126.2 (C(7)), 142.9 (C(8)), 159.5 (C(6)), 164.4 (C(4)), 165.2 (C(2)). MS (EI): 334.3 (18), 278.2 (5), 221.0 (5), 181.1 (80), 168.1 (100), 123.1 (15), 83.0 (18), 43.1 (30). HR-MS (EI): calcd. for $C_{21}H_{34}O_3$ 334.2508, found 334.2507 [M^+].

Cell proliferation assay [5]. L-929 mouse fibroblasts were obtained from the German Collection of Microorganisms and Cell cultures (DSMZ) and cultivated at 37°C and 10% CO₂ in DME medium (high glucose) supplemented with 10% fetal calf serum. Cell culture reagents came from Life Technologies Inc. (GIBCO BRL). Growth inhibition was measured in microtiterplates. Aliquots of 120 µl of the suspended cells (50.000/ml) were given to serial dilutions of the test compounds (60 µl). After 5 d, growth was determined using the MTT assay [16].

Antimicrobial assay [5]. Antimicrobial activities were determined by agar diffusion tests using paper discs of 6 mm diameter soaked with 20 µl of methanolic solution of the test compound (1 mg/ml). The organisms were grown on standard medium and seeded into liquid agar medium to a final O.D. of 0.01 (bacteria) or 0.1 (yeasts). Spores of fungi were seeded according to experience. Plates were incubated at 30°C and bacterial or fungal growth observed after 1 and 2 d.

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Table 1. *Cytotoxic and antibiotic activity of natural and unnatural derivatives 2, 3, 8, and 11*

Compd	IC ₅₀ [μg/ml] ^{a)}	Antibiotic activity ^{b)} (zone of inhibition [mm])		
		<i>Staphylococcus aureus</i>	<i>Micrococcus luteus</i>	<i>E. coli</i> tolC
(+)- 2	25	–	–	–
(–)- 2	40 ^{c)}	– ^{c)}	–	–
(–)- 3	15	+ (13)	+ (11)	–
8b	12	–	–	–
8c	13	–	–	+ (7)
11a	25	–	–	–
11a'	25 ^{c)}	– ^{c)}	–	–

^{a)} L929 fibroblasts were cultivated at 37°C and 10% CO₂ in DME medium (high glucose) with 10% fetal calf serum. Aliquots (120 μl) of suspended cells (50.000/ml) were given to serial dilutions of the respective test compound (60 μl) and growth was determined after 5 d (see Experimental part). ^{b)} Determined by an antimicrobial agar diffusion assay using 6-mm paper discs soaked with 20 μl of a methanolic solution of the respective test compound (1 mg/ml). Plates were incubated at 30°C and growth was observed after 1 and 2 d (see Experimental part). ^{c)} Data from ref. [5].