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Introduction of a Terminal Hydroxy Group into the Lipid Part of a Moenomycin-Type Transglycosylase Inhibitor Suppresses Antibiotic Activity

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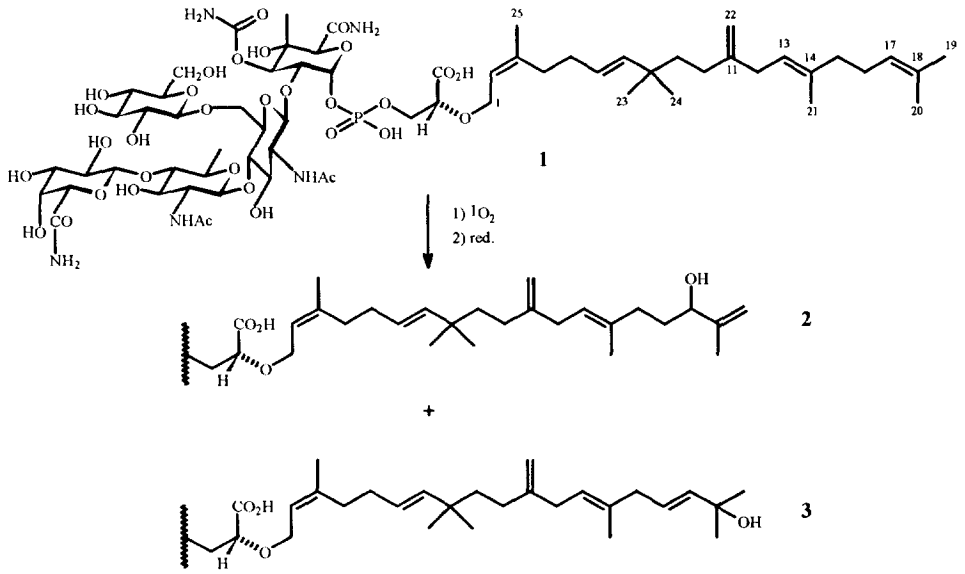
Abstract - On reaction with singlet oxygen **1** provided a mixture of **2** and **3** which could not be separated. *In-vivo*, **2/3** were antibiotically of low activity whereas in the *in-vitro* assays at least one of the compounds inhibited the transglycosylase effectively.

The final two reactions of the biosynthesis of cross-linked peptidoglycan proceeding at the extracellular surface of the cytoplasmic membrane are a transglycosylation (extending the glycan chain) and a transpeptidation (cross-linking the glycan chains through two peptide units).[1] The moenomycin-type antibiotics have been shown to be highly active and specific inhibitors of the enzyme(s) that catalyse the transglycosylation reaction. A wealth of structure-activity relations has been established in recent years. In all these studies the *in-vivo* activity against gram-positive bacteria paralleled the *in-vitro* activity with enzyme preparations obtained from *E. coli*,[2] with one notable exception: A tetrahydroxy compound obtained from **1** on reaction with osmium tetroxide (OH groups in position 11, 22, 17, and 18) showed practically full inhibitory activity in the *in-vitro* assays and was devoid of any antibiotic activity against *Staph. aureus*.[3] This very interesting observation prompted us to obtain further information on the role of polar groups in the lipid part on the transglycosylase inhibiting properties. In this paper we describe the formation, structure elucidation and antibiotic properties of two monohydroxy derivatives. Out of several reactions that were tried to modify

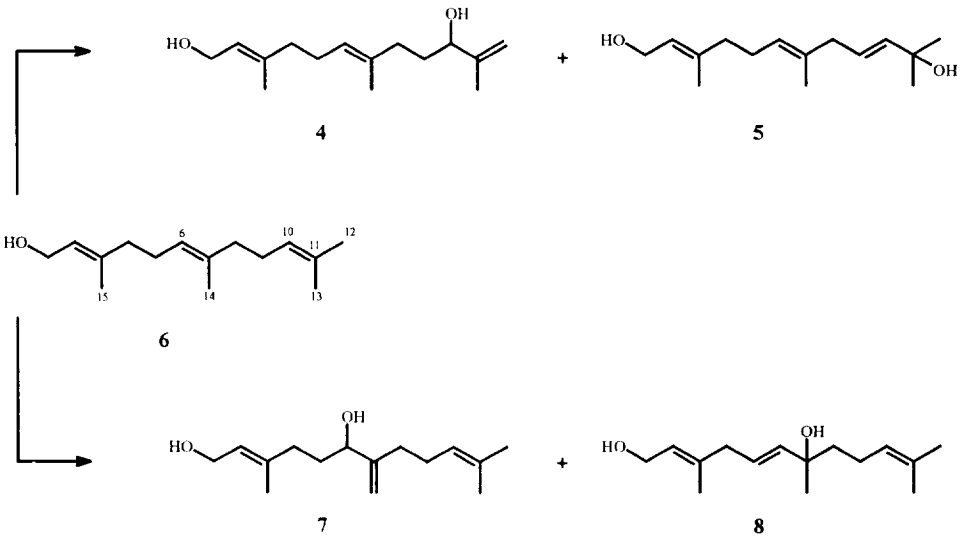
one of the olefinic units of the lipid part of **1** - oxymercuration/demercuration, reaction with hydrobromous acid, reaction with singlet oxygen - only the latter reaction led to useful results.



Thus, on exposure to singlet oxygen, followed by thiourea reduction, **1** furnished a mixture of products from which after very difficult separations one fraction (29%) could be obtained which according to the FAB and electrospray mass spectra contained only one extra oxygen (m/z = 1501.0 $[M-H]^-$ (negative ion mode), m/z = 1524.8 $[M+Na]^+$ (positive ion mode)). A second fraction (22%) contained products with two newly introduced oxygens. The first fraction was investigated in detail. The ^{13}C NMR spectra revealed that the $\Delta^{17,18}$ double bond had disappeared in the oxidation reaction. Two new olefinic carbon signals at δ = 147.8 and 112.7 were indicative of a $R_2C{=}CH_2$ group. On this basis structure **2** was proposed, and most of the ^{13}C NMR features were in accord with this structure. There remained, however, some extra signals (δ = 150.8, 140.2, 136.5, 126.1, 123.8 in the olefinic region) that could not be assigned based on structure **2**.

To solve the structural problem, in a model experiment (E,E)-farnesol (**6**) was submitted to oxidation with singlet oxygen.[4] Hydroperoxide reduction was performed with triphenylphosphine[5] since in the presence of iodide the hydroperoxides proved quite stable (^{13}C NMR signals at δ = 82-89 for C-O-OH). A GLC analysis of the reduced mixture revealed the formation of four products (2:2:1:1 ratio). One of the major components could be separated from the other oxidation products. A careful spectral analysis of this compound was fully in accord with structure **4**.[6] The other three compounds could not be separated but according to an NMR analysis the mixture clearly contained **5**[7] as major and **7**[8] and **8**[9] as minor components. Comparison of the most indicative ^{13}C NMR resonances of **4** and **5** (as collected in Table 1) with the corresponding signals of **2**

and the „extra signals" mentioned above unambigously demonstrated that the $^1O_2$ reaction product of **1** is in fact a mixture of **2** and **3**.

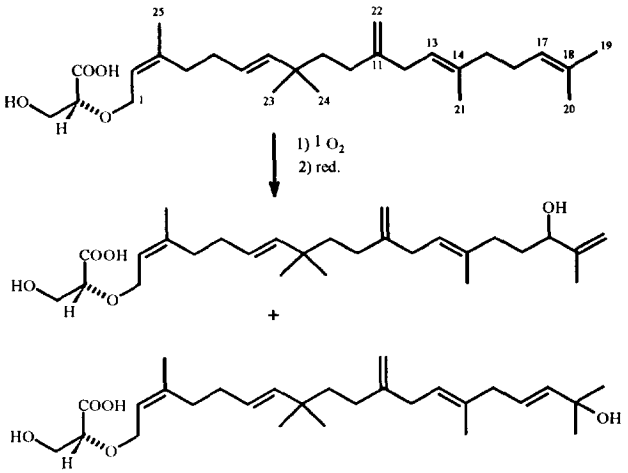


This result is in agreement with previous work on the $^1O_2$ reaction of trisubstituted isoprenic double bonds and was corroborated by submitting the moenomycin degradation product **9** $(M_A)^{10}$ to the $^1O_2$ oxidation conditions. After careful separations a fraction was obtained which according to ^{13}C NMR contained **10** and **11**. The most indicative signals are also displayed in Table 1.

Table 1. Characteristic ^{13}C chemical shifts of compounds **2**, **3**, **10**, **11**, **4**, **5**.

moenocinol numbering	**2**#	**3**#	**10**##	**11**##	**4**#	**5**##	farnesol numbering
C-18	147.8	*	149.1	71.9	148.7	71.4	C-11
C-14	137.3	136.5	137.6	136.8	136.6	135.6	C-7
C-19	112.7	29.9	111.8	30.3	112.7	30.3	C-12
C-15	36.5	43.2	37.1	43.9	36.9	43.8	C-8
C-16	33.9	126.1	34.9	126.3	34.4	125+	C-9
C-20	17.8	29.9	17.9	30.3	18.0	30.3	C-13
C-21	16.7	16.7	16.5	16.5	16.8	16.6	C-14
C-17	*	140.2	76.5	141.0	76.7	140.8	C-10

Solvent: 1:1 CD_3OD-D_2O. ## Solvent: CD_3OD.
* Hidden by sugar carbon signals. + One of several signals in this region



9

10

11

Antibiotic properties of the mixture of 2 and 3

The minimum inhibitory concentrations (MIC) of **2/3** against various microorganisms (see Table 2) have been determined by a serial two-fold agar dilution method (Müller Hinton Agar). From the results it is clear that both compounds are of significantly lower activity than **1**.

The inhibitory effect of **2/3** directly on the transglycosylation reaction was determined by the *in-vitro* assay developed earlier[11] using a crude extract from an over producer *E. coli JA200 plc19-19* and as substrate the lipid intermediate (see formula **12**) which is the immediate precursor of uncross-linked peptidoglycan. The results (see Table 3) indicate that at least one of the two products is fully active. Similar conclusions may be drawn from the figures summarized in Table 4 which were obtained with another *in-vitro* test, inhibition of the UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C]UDP-N-acetyl-glucosamine into cross-linked high-molecular weight peptidoglycan (slightly modified[12] version of the assay described by Izaki, Matsuhashi, and Strominger[13]).

Table 2. Minimum inhibitory concentrations (in μg/ml) of moenomycin degradation product **1** and a mixture of **2** and **3** against various test organisms.

test organism	**1**	**2/3**
Staph. aureus SG 511	0.025	3.125
Staph. aureus 503	0.013	3.125
Strept. pyogenes A77	0.013	0.195
Pseud. aerug. 1771m	3.125	6.25
E. coli DC 2	12.5	>25

Table 3. Effect of a mixture of **2** and **3** on the *in-vitro* formation of uncrosslinked peptidoglycan by transglycosylation.

final concentration (μg/ml)	% inhibition **2/3**
1	100
0.1	67

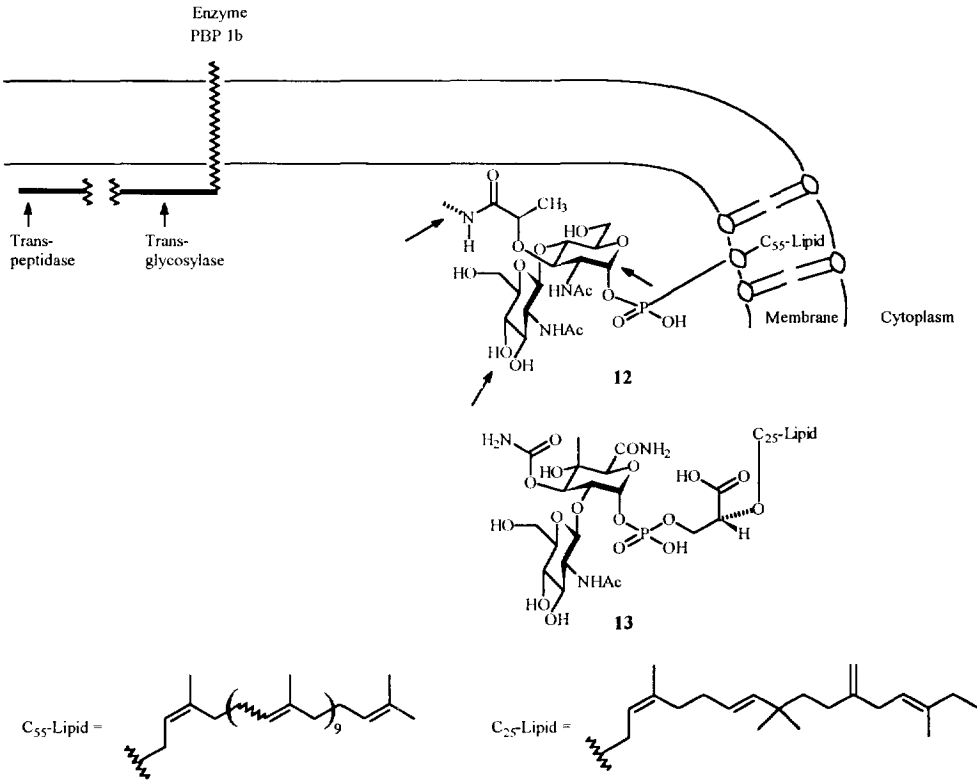
Table 4. Effect of **1** and a mixture of **2** and **3** on the *in-vitro* UDP-N-acetylmuramyl pentapeptide-dependent incorporation of [^{14}C] UDP-N-acetylglucosamine into cross-linked high-molecular weight peptidoglycan.

final concentration (μg/ml)	% inhibition **1**	**2/3**
10	85	89
1	89	89
0.1	83	83

Discussion

The minimum structural requirements for eliciting antibiotic and transglycosylase inhibiting properties have been shown to be present in moenomycin A degradation product **13**. The structural similarities with compound **12**, the last membrane precursor of peptidoglycan in *E. coli* are striking. Furthermore, moenomycin has been shown to be a competitive inhibitor of polymerase 1b.[14,15] It may be assumed that the lipid moiety attaches **12** to the membrane. Most probably the lipid part of **13** (and all moenomycin-type transglycosylase inhibitors) serves the same purpose. The glyceric acid carboxyl group of **13** may replace the first phosphate group (circle in formula **12**) of **12** when embedded into the membrane. This may explain why the methyl ester of **13** (lacking the charged carboxyl function) is antibiotically completely inactive.[2e] This hypothesis has first been put forward by Dr. M. Lampilas (Roussel Uclaf).[16] Introduction of a hydroxy group may inhibit the lipid moiety to enter the membrane lipid bilayer.[17] The results collected in Tables 3 and 4 would then mean that in the *in-vitro* assays the active site of the transglycosylase is more exposed and can be reached more readily by the more polar **2/3**.

There may, of course, also be a very trivial explanation: The moenomycin antibiotics are *in-vivo* only active against *gram-positive* bacteria. On the other hand, the *in-vitro* assays are based on enzyme preparations from *gram-negative* bacteria. The side-chain hydroxylated compounds may have structural features which allow to identify differences in the structure-activity relations of both type of enzymes. Work is in progress that is hoped to shed some light on these questions.



Experimental

General:
NMR: GEMINI 200 (Varian); GEMINI 300 (Varian), and UNITY 400 (Varian); GLC: Hewlett Packard 5890 (Series II). For other methods and instrumentation, see ref.[2a]

Reaction of 1 with $^1O_2$

A solution of **1** (99.6 mg, 0.0670 mmol) and Rose Bengal (1.0 mg) in dry methanol (30 ml) was irradiated for 5 h at 20°C with an external 1000 W halogen lamp while passing continously a stream of oxygen saturated with methanol through the solution. Hydroperoxides were reduced with thiourea (49.7 mg, 0.6529 mmol). After solvent evaporation repeated chromatographic separations (LC, SiO_2, $CHCl_3$-methanol-water 18:11:2.7; HPLC, RP18, ethanol-water 1:1.5; HPLC, RP18, ethanol-water-CH_3CN 1:8:1) furnished a mixture (29.1 mg, 29%) of **2** and **3** and a fraction of dihydroxylated derivatives of **1** (22.8 mg, 22%).

2-O-{2-Acetylamino-4-O-[2-acetylamino-4-O-((5S)-5-carbamoyl-β-L-arabinopyranosyl)-2, 6-dideoxy-β-D-glucopyranosyl]-2-deoxy-6-O-β-D-glucopyranosyl-β-D-glucopyranosyl}-3-O-carbamoyl-1-O-{[(R)-2-carboxy-2-((2Z, 6E, 13E)-17-hydroxy-3, 8, 8, 14, 18-pentamethyl-11-methylene-nonadeca-2, 6, 13, 18-tetraene-1-yloxy)-ethoxy]-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronamide (2)

The spectra were taken from an unseparable mixture of **2** and **3** (roughly 1:1, according to NMR). Collected are all carbon signals with identical chemical shifts for **2** and **3** as well as the characteristic signals of **2** that were used for the structure elucidation (see also Table 1). The mass spectra also refer to the mixture.- ^{13}C NMR (100.6 MHz, CD_3OD-D_2O 1:1, DEPT): δ = 175.2, 174.8, 174.1, 173.8 ($NHCOCH_3^{C, E}$, C-$6^{B, F}$); 159.1 ($OCONH_2^F$); 150.7 (C-11^I); 147.8 (C-18^I); 142.0 (C-3^I); 141.3 (C-7^I); 137.3 (C-14^I); 126.8 (C-6^I); 123.2 (C-13^I); 122.2 (C-2^I); 112.7 (C-19^I); 109.9 (C-22^I); 104.3, 103.8, 103.2, 102.4 (C-$1^{B, C, D, E}$); 95.5 (C-1^F); 84.1, 81.2 (C-4^C, C-4^E); 77.6-67.0 (signals not assigned); 62.0 (C-6^D); 56.6, 56.2 (C-$2^{C, E}$); 42.5 (C-9^I); 36.5 (C-15^I); 36.3 (C-8^I); 36.0 (C-12^I); 33.9 (C-16^I); 33.0, 32.4 (C-5^I, C-4^I); 32.0 (C-10^I); 28.0 (C-23^I, C-24^I); 24.3 (C-25^I); 17.8 (C-20^I); 16.7 (C-21^I).- $C_{64}H_{104}N_5O_{33}P$ (1502.52, 1501.64), FAB MS[18] (matrix: lactic acid): m/z = 1562.2 $[M+Na+K-H]^+$; 1540.2 $[M+K]^+$; 1524.2 $[M+Na]^+$; 982.2 $[f+Na-H]^+$.- ES MS (negative ion mode, cone voltage 45): m/z = 1501.0 $[M-H]^-$; 750.0 $[M-2H]^{2-}$; (cone voltage 100): m/z = 1056.1 [g]$^-$; 1013.3 [g-(HNCO)]$^-$; 838.3 [g-(HNCO)-B]$^-$; 728.5 $[M-(HNCO)-2H]^{2-}$.- ES MS (positive ion mode): m/z = 1540.7 $[M+K]^+$; 1524.8 $[M+Na]^+$; 1146 $[h]^+$; 960.4 $[f]^+$; 798.4 $[f-D]^+$; 728.5 $[e]^+$; 566.4 $[e-D]^+$; 363.3 $[c]^+$.

2-O-{2-Acetylamino-4-O-[2-acetylamino-4-O-((5S)-5-carbamoyl-β-L-arabinopyranosyl)-2,6-dideoxy-β-D-glucopyranosyl]-2-deoxy-6-O-β-D-glucopyranosyl-β-D-glucopyranosyl}-3-O-carbamoyl-1-O-{[(R)-2-carboxy-2-((2Z, 6E, 13E, 16E)-18-hydroxy-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2, 6, 13, 16-tetraene-1-yloxy)-ethoxy]-hydroxyphosphoryl}-4-C-methyl-α-D-glucopyranuronamide (3)

^{13}C NMR (100.6 MHz, CD_3OD-D_2O 1:1, DEPT, characteristic signals of **3** obtained from the spectra of the mixture of **2** and **3**): δ = 150.8 (C-11^I); 140.2 (C-17^I); 136.5 (C-14^I); 126.1 (C-16^I); 123.8 (C-13^I); 109.8 (C-22^I); 43.2 (C-15^I); 29.9 (C-19^I, C-20^I); 16.7 (C-21^I).[19]

Dihydroxy derivative(s) of 1

^{13}C NMR (100 MHz, CD_3OD-D_2O 1:1, DEPT): δ = 177.7 (C-1^H); 175.4, 175.0, 174.3, 174.1 ($NHCOCH_3^{C, E}$, C-6$^{B, F}$); 159.4 ($OCONH_2^F$); 152.3, 151.4, 142.3, 141.7, 140.7, 136.5, 130.5, 127.1, 126.0, 122.3, 112.7, 112.2 (olefinic carbon signals); 104.5, 104.1, 103.3, 102.7 (C-1$^{B, C, D, E}$); 95.8 (C-1^F); 84.4, 81.7 (C-4^C, C-4^E); 81.1-67.1 (signals not assigned); 62.3 (C-6^D); 56.8, 56.7 (C-2$^{C, E}$); 43.6-16.2 (signals not assigned).- $C_{64}H_{104}N_5O_{34}P$ (1518.52, 1517.63). ES MS (negative ion mode, cone voltage 100): m/z = 1517.2 $[M-H]^-$; 1013.5 [g-(HNCO)]$^-$; 838.5 [g-(HNCO)-B]$^-$; 736.6 $[M-(HNCO)-2H]^{2-}$; (cone voltage 45): m/z = 758.0 $[M-2H]^{2-}$.- ES MS (positive ion mode): m/z = 1556.6 $[M+K]^+$; 1541.1 $[M+Na]^+$; 1146.4 [h]$^+$; 960.4 [f]$^+$; 728.5 [e]$^+$; 566.3 [e-D]$^+$; 363.2 [c]$^+$.[18]

(E,E)-Farnesol [(3E,7E)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol] (6)

(E,E)-farnesol was purified by LC, petrolether-ethyl acetate 10:1. ^1H NMR[20] (300 MHz, $CDCl_3$, H,H COSY, C,H COSY): δ = 1.61 (s, 6H) and 1.69 (s, 6H, CH_3-12, CH_3-14, CH_3-13, CH_3-15); 2.01 (m, 2H, CH_2-4); 2.05 (m, 2H, CH_2-9); 2.08 (m, 2H, CH_2-8); 2.11 (m, 2H, CH_2-5); 3.65 (1H, OH); 4.16 (d, 2H, CH_2-1, J = 7 Hz); 5.09 (m, 1H, 10-H); 5.12 (m, 1H, 6-H); 5.43 (dt, 1H, 2-H, ^3J = 7 Hz, ^4J = 1 Hz).- ^{13}C NMR ($CDCl_3$, DEPT): δ = 140.2 (C-3); 135.8 (C-7); 131.8 (C-11); 124.8 (C-10); 124.3 (C-6); 123.9 (C-2); 59.8 (C-1); 40.2 (C-4); 40.0 (C-8); 27.2 (C-9); 26.8 (C-5); 26.2 (C-12); 18.2 (C-13); 16.7 and 16.5 (C-14, C-15).

Photooxygenation of (E,E)-farnesol (6)

A solution of **6** (192.0 mg, 0.86 mmol) and tetraphenylporphin (3.5 mg) in CH_2Cl_2 (50 ml) was irradiated for 5 min at 0°C with an external 1000 W halogen lamp while passing continously a stream of oxygen saturated with CH_2Cl_2 through the solution. The reaction mixture was then transferred into a reaction flask, PPh_3 (340 mg, 1.30 mmol) was added, and the mixture was stirred at 20°C for 2h. After solvent evaporation the residue was filtered through silica gel (petrolether-ethyl acetate 3:1) to remove the sensitizer, PPh_3, $OPPh_3$, and not consumed farnesol (48.6 mg). After solvent evaporation the mixture of the oxidation products (122.7 mg) was shown by GLC (25 m x 0.2 mm glass capillary column, cross-linked methylsilicone, 160°C, carrier gas: H_2) to

contain **5** (retention time: 19.9 min, 31%), **4** (25.5 min, 31%), **7** and **8** (21.6 min and 25.8 min, each 17%). MPLC (petrolether-ethyl acetate-^{t}butyl methyl ether 12:5:5) furnished pure **4**; **5** was enriched whereas **7** and **8** could not be separated.

(2E,6E)-3,7,11-Trimethyl-2,6,11-dodecatriene-1,10-diol (4)

^{1}H NMR (300 MHz, $CDCl_3$, H,H COSY): δ = 1.65 (m, 2H, CH_2-9, identified by H,H COSY); 1.67 (s, 3H), 1.73 (s, 3H) and 1.63 (s, 3H, CH_3-14, CH_3-13, CH_3-15); 2.15 (m, 2H) and 2.07 (m, 4H, CH_2-4, CH_2-5, CH_2-8); 4.05 (t, 1H, 10-H); 4.14 (d, 2H, J = 7 Hz, CH_2-1); 4.84 (s, 1H, 12-H_{trans}); 4.94 (s, 1H, 12-H_{cis}); 5.17 (t, 1H, 6-H); 5.41 (t, 1H, 2-H).- ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 147.9 (C-11); 139.1 (C-3); 135.5 (C-7); 124.9 (C-6); 124.5 (C-2); 111.3 (C-12); 75.7 (C-10); 59.6 (C-1); 39.9 (C-4); 36.2 (C-8); 33.3 (C-9); 26.4 (C-5); 18.1 (C-13); 16.5 and 16.4 (C-14, C-15).- ^{13}C-NMR (50.3 MHz, D_2O-CD_3OD 1:1): δ = 148.7 (C-11); 140.7 (C-3); 136.6 (C-7); 125.7 (C-6); 124.7 (C-2); 112.7 (C-12); 76.7 (C-10); 59.7 (C-1); 40.9 (C-4); 36.9 (C-8); 34.4 (C-9); 27.5 (C-5); 18.0 (C-13); 16.7 and 16.8 (C-14, C-15).- $C_{15}H_{26}O_2$ (238.37, 238.19).- FAB MS (matrix: lactic acid): m/z = 261.2 $[M+Na]^+$; 221.2 $[M+H-H_2O]^+$; 203.2 $[M+H-2H_2O]^+$.

(2E,6E,9E)-3,7,11-Trimethyl-2,6,9-dodecatriene-1,11-diol (5)

^{1}H NMR[20] (200 MHz, $CDCl_3$, homonuclear decoupling, H,H COSY): δ = 1.30 (s, 6 H, CH_3-12, CH_3-13); 1.57 (s, 3H) and 1.66 (s, 3H, CH_3-14, CH_3-15); 2.04-2.14 (m, 4H, CH_2-5, CH_2-4); 2.65 (d, 2H, CH_2-8, $J_{8,9}$ = 4.1 Hz); 2.74 (broad signal, 1H, OH); 4.13 (d, 2H, CH_2-1, $J_{1,2}$ = 7.0 Hz); 5.11 (m, 1H, 6-H, $J_{5,6}$ = 6.6 Hz); 5.39 (t, 1H, 2-H); 5.58-5.61 (m, 2H, 9-H, 10-H).- ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 139.7 (C-10); 138.7 (C-3); 134.5 (C-7); 125.5 (C-9); 125.2 (C-6); 124.5 (C-2); 70.9 (C-11); 59.5 (C-1); 42.7 (C-8); 39.8 (C-4); 30.2 (C-12, C-13); 26.5 (C-5); 16.6 (C-14, C-15). For characteristic signals from the CD_3OD solution spectra, see Table 1.

(2E, 10)-7-Methylene-3,11-Dimethyl-2,10-dodecadiene-1,6-diol (7)

^{13}C NMR[21] (50.3 MHz, $CDCl_3$): δ = 152.0 (C-7); 139.1 (C-3); 132.1 (C-11); 125.0, 124.3 (C-2, C-10); 109.9 (C-14); 75.1 (C-6); 59.5 (C-1); 36.0, 33.8, 31.8 (C-4, C-5, C-8); 27.0 (C-9); 26.1 (C-12); 18.1 (C-13); 16.7 (C-15).

(2E, 5E, 10)-3,7,11-Trimethyl-2,5,10-dodecatriene-1,7-diol (8)

^{13}C NMR[21] (50.3 MHz, $CDCl_3$): δ = 139.3 (C-6); 138.0 (C-3); 132.0 (C-11); 125.6, 125.0, 124.3 (C-5, C-2, C-10); 73.2 (C-7); 59.5 (C-1); 43.0, 42.7 (C-4, C-8); 28.4 (C-14); 26.0 (C-12); 23.4 (C-9); 18.1 (C-13); 16.7 (C-15).

(R)-3-Hydroxy-2-{(2Z,6E,13E)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2,6,13,17-tetraene-1-yloxy}-propanoic acid (M_A, 9)

9 was purified by LC (SiO_2, $CHCl_3$-methanol-acetic acid 20:1:0.5).- ^{13}C NMR (50.3 MHz, CD_3OD): δ = 175.2 (broad signal, C-1^H); 151.3 (C-11^I); 142.2 (C-3^I); 142.0 (C-7^I); 137.6 (C-14^I); 132.5 (C-18^I); 127.07 (C-6^I); 125.7 (C-17^I); 123.8 (C-13^I); 123.0 (C-2^I); 109.6 (C-22^I); 80.8 (broad signal, C-2^H); 68.0 (C-1^I); 64.3 (C-3^H); 43.2 (C-9^I); 41.2 (C-15^I); 36.8 (C-8^I); 36.3 (C-12^I); 33.7, 32.8 (C-5^I, C-4^I); 32.7 (C-10^I); 28.2 (C-23^I, C-24^I); 28.0 (C-16^I); 26.3 (C-20^I); 24.2 (C-25^I); 18.2 (C-19^I); 16.5 (C-21^I).- ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 150.5 (C-11^I); 142.6 (C-3^I); 141.2 (C-7^I); 136.8 (C-14^I); 131.8 (C-18^I); 125.6 (C-6^I); 124.8 (C-17^I); 122.5 (C-13^I); 121.0 (C-2^I); 108.9 (C-22^I); 80.6 (broad signal, C-2^H); 67.5 (C-1^I); 63.5 (C-3^H); 41.9 (C-9^I); 40.2 (C-15^I); 36.0 (C-8^I); 35.4 (C-12^I); 32.9, 31.9 (C-5^I, C-4^I); 31.9 (C-10^I); 27.7 (C-23^I,C-24^I); 27.1 (C-16^I); 26.2 (C-20^I); 24.1 (C-25^I); 18.2 (C-19^I); 16.4 (C-21^I).

Photooxygenation of (R)-3-Hydroxy-2-{(2Z, 6E, 13E)-3,8,8,14,18-pentamethyl-11-methylene-nonadeca-2, 6, 13, 17-tetraene-1-yloxy}-propanoic acid (M_A, 9)

A solution of **9** (220.0 mg, 0.492 mmol) and methylene blue in CH_2Cl_2 (50 ml) was irradiated for 5 min (slightly too long, TLC control, $CHCl_3$-methanol-acetic acid 15:2:0.5) at 0°C with an external 1000 W halogen lamp while passing continously a stream of oxygen saturated with CH_2Cl_2 through the solution. The reaction mixture was then transferred into a reaction flask, PPh_3 (325.9 mg, 1.24 mmol) was added, and the mixture was stirred at 20°C for 1h. After solvent evaporation the residue was separated by LC ($CHCl_3$-methanol-acetic acid 400:20:0.5) to give **10** (in the first fractions, 22.2 mg, 0.047 mmol), a mixture of **10** and **11** (42.3 mg, 0.093 mmol), and a fraction containing dihydroxylated derivatives of **9**. Almost pure **10** and **11** were obtained by repeated LC. The ratio of **10**:**11** in the reaction mixture was 1:1 (estimated from the ^{13}C NMR spectrum of the mixture).

(R)- 3-Hydroxy-2-{(2Z, 6E, 13E)-17-hydroxy-3, 8, 8, 14, 18-pentamethyl-11-methylene-nonadeca-2, 6, 13, 18-tetraene-1-yloxy}-propanoic acid (10)

The sample was contaminated with some **11**. ^1H NMR (200 MHz, CD_3OD, homonuclear decoupling): δ = 0.98 (s, 6H, CH_3-23^I, CH_3-24^I); 1.34-1.43 (m, 2H, CH_2-9^I); 1.61 (m, 2H, CH_2-16^I) [22]; 1.64 (s, 3H, CH_3-21^I); 1.72 (s, 3H, CH_3-20^I); 1.76 (s, 3H, CH_3-25^I); 1.88-2.16 (8H, CH_2-10^I, CH_2-4^I, CH_2-5^I, CH_2-15^I); 2.72 (d, J = 6 Hz, CH_2-12^I); 3.78-4.20 (6H, 2^H-H, CH_2-1^I, 17^I-H, CH_2-3^H); 4.68 (s, 2H, CH_2-22^I); 4.82 and 4.92 (2 s, 2H, CH_2-20^I); 5.20 (m, 1H, 13^I-H); 5.30-5.50 (3H, 6^I-H, 7^I-H, 2^I-H).- ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 150.4 (C-11^I); 147.7 (C-18^I); 142.4 (C-3^I); 141.1 (C-7^I); 136.5 (C-14^I); 125.7 (C-6^I); 123.0 (C-13^I); 121.1 (C-2^I); 111.7 (C-19^I); 109.1 (C-22^I); 76.2 (C-17^I); 67.1 (broad signal, C-1^I); 63.2 (broad signal, C-3^H); 41.8 (C-9^I); 36.1 (C-15^I); 36.0 (C-8^I); 35.8 (C-12^I); 33.6 (C-16^I); 32.9, 31.8 (C-5^I, C-4^I); 31.6 (C-10^I); 27.8 (C-23^I, C-24^I);

24.0 (C-25^{I}); 18.0 (C-20^{I}); 16.5 (C-21^{I}).- ^{13}C NMR (50.3 MHz, CD_3OD): δ = 175.8 (broad signal, C-1^{H}); 151.4 (C-11^{I}); 149.1 (C-18^{I}); 142.2 (C-3^{I}); 141.9 (C-7^{I}); 137.6 (C-14^{I}); 127.1 (C-6^{I}); 123.8 (C-13^{I}); 123.0 (C-2^{I}); 111.8 (C-19^{I}); 109.6 (C-22^{I}); 80.6 (broad signal, C-2^{H}); 76.5 (C-17^{I}); 67.8 (C-1^{I}); 64.3 (C-3^{H}); 43.1 (C-9^{I}); 37.1 (C-15^{I}); 36.7 (C-8^{I}); 36.2 (C-12^{I}); 34.9 (C-16^{I}); 33.7, 32.8 (C-5^{I}, C-4^{I}); 32.6 (C-10^{I}); 28.1 (C-23^{I}, C-24^{I}); 24.1 (C-25^{I}); 17.9 (C-20^{I}); 16.5 (C-21^{I}).- $C_{28}H_{46}O_5$ (462.67, 462.33), FAB MS (matrix: lactic acid): m/z = 523.3 $[M+Na+K-H]^+$; 507.3 $[M+Na+Na-H]^+$; 485.3 [M+Na].

(R)-3-Hydroxy 2-{(2Z, 6E, 13E, 16E)-18-hydroxy-3, 8, 8, 14, 18-pentamethyl-11-methylene-nonadeca-2, 6, 13, 16-tetraene-1-yloxy}-propanoic acid (11)

The sample was contaminated with some **10**.- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (s, 6H, CH_3-23^{I}, CH_3-24^{I}); 1.24 (s, 6H, CH_3-19^{I}, CH_3-20^{I}); 1.27-1.32 (m, 2H, CH_2-9^{I}); 1.51 (s, 3H, CH_3-21^{I}); 1.65 (s, 3H, CH_3-25^{I}); 1.79-1.83 (2H, CH_2-10^{I}); 1.95-2.05 (4H, CH_2-4^{I}, CH_2-5^{I}); 2.62-2.64 (m, 4H, CH_2-12^{I}, CH_2-15^{I}); 3.70-4.20 (5H, 2^{H}-H, CH_2-1^{H}, CH_2-1^{I}); 4.61 (s, 2H, CH_2-22^{I}); 5.10-5.30 (4H, 13^{I}-H, 6^{I}-H, 7^{I}-H, 2^{I}-H); 5.50-5.55 (2H, 16^{I}-H, 17^{I}-H).- ^{1}H NMR (300 MHz, CD_3OD, H,H COSY, C,H COSY, homonuclear decoupling): δ= 0.96 (s, 6H, CH_3-23^{I}, CH_3-24^{I}); 1.25 (s, 6H, CH_3-19^{I}, CH_3-20^{I}); 1.34-1.40 (m, 2H, CH_2-9^{I}); 1.60 (s, 3H, CH_3-21^{I}); 1.75 (s, 3H, CH_3-25^{I}); 1.87-1.93 (m, 2H, CH_2-10^{I}); 2.05-2.15 (4H, CH_2-4^{I}, CH_2-5^{I}); 2.69-2.71 (m, 4H, CH_2-12^{I}, CH_2-15^{I}); 3.71-3.87 (3H, 2^{H}-H, CH_2-1^{H}); 4.08 and 4.22 (2H, CH_2-1^{I}); 4.66 (s, 2H, CH_2-22^{I}); 5.17-5.21 (m, 1H, 13^{I}-H); 5.29-5.41 (m, 3H, 6^{I}-H, 7^{I}-H, 2^{I}-H); 5.58-5.60 (2H, 16^{I}-H, 17^{I}-H).- ^{13}C NMR (50.3 MHz, CD_3OD, C,H COSY, DEPT): δ = 176.5 (C-1^{H}), 151.3 (C-11^{I}); 142.2 (C-3^{I}); 142.0 (C-7^{I}); 141.0 (C-17^{I}); 136.8 (C-14^{I}); 127.1 (C-6^{I}); 126.3 (C-16^{I}); 124.4 (C-13^{I}); 123.0 (C-2^{I}); 109.6 (C-22^{I}); 80.9 (broad signal, C-2^{H}); 71.9 (C-18^{I}); 67.8 (C-1^{I}); 64.3 (C-3^{H}); 43.9 (C-15^{I}); 43.1 (C-9^{I}); 36.8 (C-8^{I}); 36.3 (C-12^{I}); 33.7, 32.8 (C-5^{I}, C-4^{I}); 32.7 (C-10^{I}); 30.3 (C-19^{I}, C-20^{I}); 28.1 (C-23^{I}, C-24^{I}); 24.1 (C-25^{I}); 16.5 (C-21^{I}).- $C_{28}H_{46}O_5$ (462.67, 462.33), FAB MS (matrix: lactic acid): m/z = 523.3 $[M+Na+K-H]^+$; 507.3 $[M+Na+Na-H]^+$; 485.3 $[M+Na]^+$.

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References and Notes

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16 We wish to thank Dr. M. Lampilas for many fruitful discussions on this point.

17 Or arrest it in a wrong position.

18 For the nomenclature of the fragment ions, see ref.[2e]

19 The C-21 signals of **2** and **3** were overlapping.

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21 The signals at δ = 152.0, 109.9, 75.1 are characteristic of **7** and those at 139.3, 73.2, 28.4 of **8**. All other signals have been assigned based on increments and on structurally similar compounds and have possibly in some cases to be interchanged between **7** and **8**, see refs. [8,9]

22 The signal of CH_2-16 was overlapping with the methyl signals.

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