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Synthesis of a Stereoisomeric Mixture of (±)-PM-Toxin B, a Host-specific Corn Pathotoxin Produced by *Phyllosticta maydis*

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A stereoisomeric mixture of (±)-PM-toxin B [6,14,22,30,32-pentahydroxy-8,16,24-trioxotritriacontane] has been synthesized *via* three steps of Grignard addition and shows virtually the same biological activities as the natural PM-toxin B.

PM-toxin,[1] isolated from the corn pathogen, *Phyllosticta maydis*, selectively damages only corn with Texas-male sterile cytoplasm at 10^{-9} to 10^{-8} M, but not corn with normal fertile cytoplasm at 10^{-5} M. The toxin is a mixture of biogenetically related compounds of apparently equal toxicity. The four major components have been purified and characterized, although the stereochemistry of the hydroxyl functions was not determined.[2]

We report here the first synthesis of one of the major components (PM-toxin B) as a mixture of stereoisomers possessing the *syn*-1,3-hydroxy configuration at C-30 and C-32. Synthetic PM-toxin B has the same specific toxicity toward corn and thus the proposed structure of the natural toxin is confirmed.

Our synthetic outline is shown in Fig. 1. C–C bond formations at C-8, C-16 and C-24 in PM-toxin B were chosen because of their applicability to chiral synthesis by using precursors like β-hydroxyesters which are readily prepared by unsymmetrical reduction of β-ketoesters with baker's yeast. Synthesis of chiral PM-toxin B with a variety of known absolute stereochemistries can not only establish the absolute stereochemistry of the natural toxin, but also may lead to a better understanding of the mode of action for PM-toxins.

Three segments (**a**, **b**, **c**) corresponding to the C1～8, C9～16 and C17～24, and C25～33 units in PM-toxin B were initially synthesized as shown in Scheme 1. The synthesis of segment *a* (**3**) started from β-hydroxyoctanoate **1**. Etherification[3] of **1** with dimethoxymethane and phosphorous pentoxide in chloroform, and the reduction of **2** with diisobutylaluminum hydride in *n*-hexane (−78°C, 45 min) gave the desired segment **a** (**3**) in a 46.9% overall yield.

The synthesis of segments **b** and **c** was designed to utilize the convenient monoal-

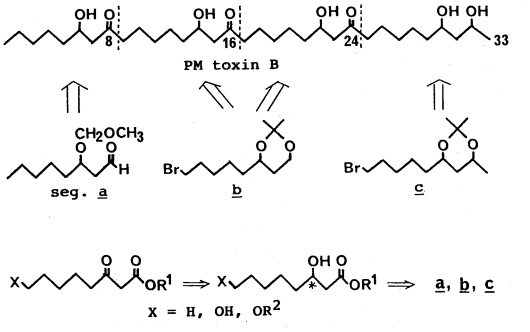
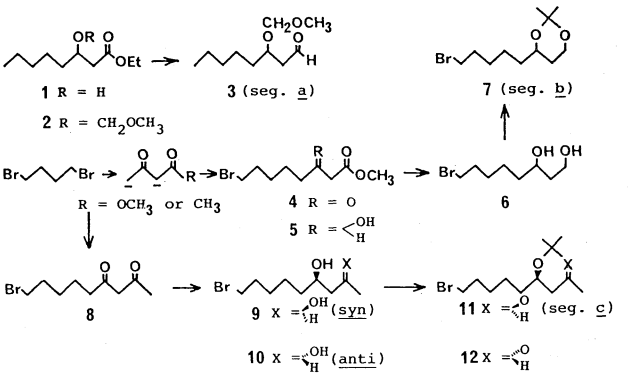


FIG. 1. Synthetic Outline of (±)-PM-Toxin B.



SCHEME 1. Synthetic Route of Segments **a**, **b**, **c**.

kylation of the dianion of the β-ketoester and β-diketone with 1,4-dibromobutane.[4] Condensation of the dianion generated from methyl acetoacetate with 1.5 equiv. of 1,4-dibromobutane (0°C, 10 min) gave the desired β-ketobromoester **4** in a 52.5% yield. Reduction of **4** with sodium borohydride in methanol (0°C, 30 min) produced the β-hydroxy bromoester **5**, which on reduction with 3 equiv. of diisobutylaluminum hydride in tetrahydrofuran (0°C, 1 hr) gave the β-dihydroxybromide **6**. Acetonization of **6** with 2,2-dimethoxypropane and *p*-toluenensulfonic acid in dioxane (20°C, 1 hr) afforded the desired segment **b** (**7**) in a 63.1% overall yield. Direct conversion of **4** to **6** by reduction with an excess of diisobutylaluminum hydride was unsatisfactory because a considerable amount of **4** invariably remained unreacted.

Alkylation of the dianion of 2,4-pentanedione to 1,4-dibromobutane (1.5 equiv.) gave the desired β-diketo-bromide **8**, which reacted with sodium borohydride in methanol (10°C, 30 min) to provide a chromatographically separable mixture of the *syn* and *anti* isomers (**9** and **10**) in 10.8% and 37.0% yields, respectively. Acetonization of **9** and **10** gave the desired segment *c* (**11**) and its isomer (**12**), respectively.

The relative configurations of **9** and **10** were determined on the basis of NOE measurements for each benzylideneacetal, **13** and **14** (Fig. 2). Enhancement of both the C-6H and C-8H signals upon irradiation of benzylic H in

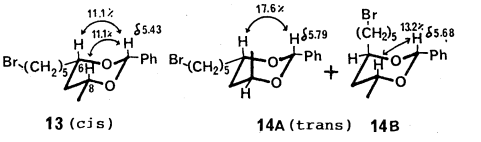
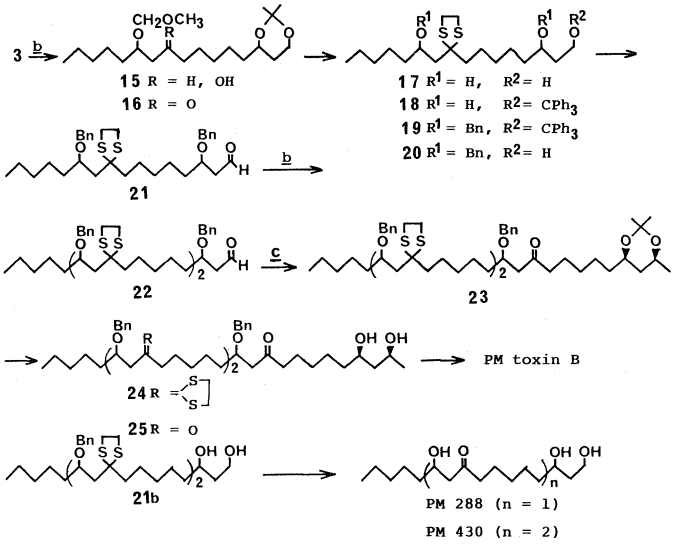


FIG 2. NOE Experiments of the Benzylideneacetals of **9** and **10** (400 MHz, C_6D_6).

TABLE I. ^1H- AND ^{13}C-NMR DATA FOR THE PENTAACETATE OF PM-TOXIN B AND THE DIACETATE OF **9** and **10**

	Chemical shift ($^1H/^{13}C$ ppm) in $CDCl_3$	
	C-6 (C-30)	C-8 (C-32)
PM toxin B	4.93/71.2	4.93/68.1
syn	4.93/71.1	4.93/68.1
anti	4.96/70.3	4.96/67.1

13 (derived from the less polar isomer **9**) suggested that C-6H, C-8H and benzylic H should be *syn* diaxial. To the contrary, another benzylidene isomer **14** (derived from the more polar isomer **10**) consisted of two diastereoisomers, and the expected NOE was observed at either the C-6H or C-8H signal in each isomer. These results show clearly that **13** has the *cis* configuration and **14A** and **14B** have *trans* configuration. The *syn* stereochemistry for the β-diol moiety in PM-toxin B was



SCHEME 2. Synthetic Route of (±)-PM-Toxin B.

further elucidated by ^{1}H- and ^{13}C-NMR comparisons with the pentaacetate of PM-toxin B, and the diacetates of the 1,3-*syn* -diol **9** and the *anti*-diol **10** (Table I). Therefore, the *cis*-acetonide **11**, which was derived from the minor product **9** possessing the same *syn* stereochemistry as the natural PM-toxin-B, was used as the segment **c**.

The reactions for coupling each segment, leading to PM-toxin B, were carried out as shown in Scheme 2. The reaction of **3** with the Grignard reagent generated from **7** in tetrahydrofuran (−10 to −5°C, 10 min) produced the alcohol **15** in a 94.0% yield. Oxidation[5] of **15** with pyridinium dichromate in *N*,*N*-dimethylformamide (rt, 18 hr) afforded the corresponding ketone **16** (69.6%), which reacted with 4 equiv. of ethanedithiol and boron trifluoride etherate in dichloromethane (t_R, 18 hr)[6] to give the dithioacetal **17** (62.8%) with deprotection of the isopropylideneacetal and methoxymethyl ether groups.

Selective tritylation[7] of **17** with 1.0 equiv. of trityl chloride, 4-dimethylaminopyridine and triethylamine in dichloromethane (t_R, 18 hr) gave the monotrityl ether **18** (96.9%). Benzylation[8] of **18** with benzyl bromide and sodium hydride in tetrahydrofuran (t_R, 2 day) followed by hydrolysis of **19** with *p*-toluenesulfonic acid in methanol and chloroform (20°C, 2 hr) afforded the dibenzyl ether **20** in a 39.9% overall yield. Corrins oxidation yielded the corresponding aldehyde **21** in a 50.5% yield. The C_{16}-aldehyde **21** was converted to the homologous C_{24}-aldehyde **22** in the same reaction sequence described above. A final Grignard reaction of the C_{24}-aldehyde **22** with the segment **c** (**11**) followed by Corrins oxidation gave the C_{33}-acetonide **23**, which was deacetonized with aq. acetic acid (50°C, 5 hr) and then dethioacetalized with mercuric chloride and mercuric oxide in aq. acetonitrile (t_R, 4 hr) to give the tribenzyl ether **25** in a 46.5% yield from **23**. Finally, debenzylation by hydrogenolysis using an excess of activated palladium black[9] as a catalyst in a mixed solvent (acetic acid–dioxane–water = 4 : 1 : 1) at 35°C for 24 hr gave the desired stereoisomeric mixture of (±)-PM-toxin B (37.0% yield), which was identical with natural PM-toxin B by comparison of their FD-MS, 400 MHz ^{1}H-NMR and 22.5 MHz ^{13}C-NMR (Figs. 3 and 4), and behavior with TLC and HPLC. Two shorter chain versions of PM-toxin B, PM-288 (C_{16}) and PM-430 (C_{24}), were also prepared from each protected compound (**17** and **21b**).

The biological activities of the synthetic

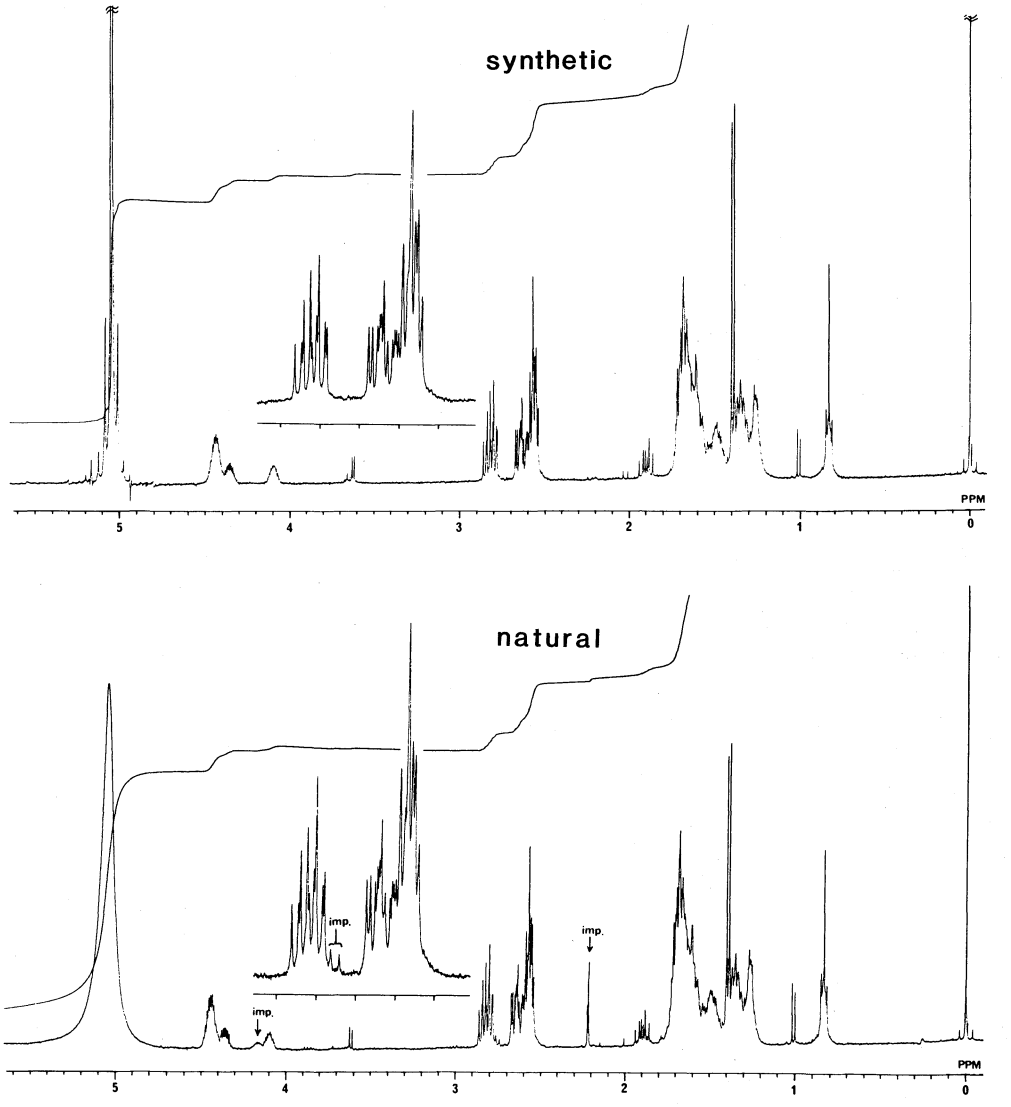


FIG. 3. 400 MHz ^{1}H-NMR Spectra of Synthetic and Natural PM-Toxin B in d_5-Pyridine.

PM-toxin B and analogs were evaluated using assays of the inhibition of dark CO_2 fixation by thin leaf slices and stimulation of NADH oxidation in mitochondria as described previously.[10] The biological activity of synthetic (±)-PM-toxin B was indistinguishable from that of natural PM-toxin in both assays on susceptible (W64AT) corn (Table II, Fig. 5), providing further evidence that the proposed structure for PM-toxin B[1,2] is correct. In addition, the fact that the biological activity of the stereoisomeric mixture of synthetic (±)-PM-toxin B is equal to that of natural PM-toxin B suggests that the stereochemistry may not be important for maximum biological activity.

The shorter analogs of PM-toxin B (PM-430 and PM-288) were less active on susceptible corn than PM-toxin B itself. PM-430 was about an order of magnitude less active and PM-288 was about three orders of magnitude less active than PM-toxin B itself.

As with analogs of HMT-toxin examined earlier,[10] chain length appears to be important

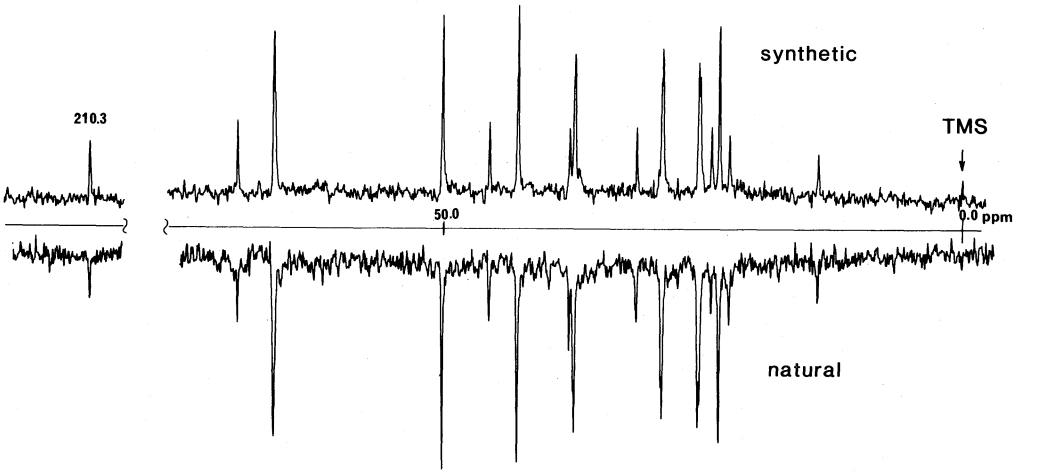


FIG. 4. 22.5 MHz ^{13}C-NMR Spectra (PND) of Synthetic and Natural PM-Toxin B in d_5-Pyridine.

TABLE II. EFFECTS OF NATURAL AND SYNTHETIC PM-TOXIN B AND ITS SHORTER ANALOGS ON THE INHIBITION OF DARK CO_2 FIXATION BY LEAF SLICES OF SUSCEPTIBLE (W64AT) CORN

Compound	Concentration (mol/liter)									
	3×10^{-9}	1×10^{-8}	3×10^{-8}	1×10^{-7}	3×10^{-7}	1×10^{-6}	3×10^{-6}	1×10^{-5}	3×10^{-5}	1×10^{-4}
	% Inhibition[a]									
PMB(natural)	15	21	29	33	52					
(±)PMB(synthetic)	12	23	31	38	46					
PM 430			4	22	31	40	45			
PM 288						−6[b]	7	37	44	51

[a] Averages of two experiments. Control rates of CO_2 fixation averaged 17.4 ± 1.8 nmol CO_2 slice^{-1} hr^{-1}. Standard deviations averaged 4%.

[b] Minus indicates rates above control values.

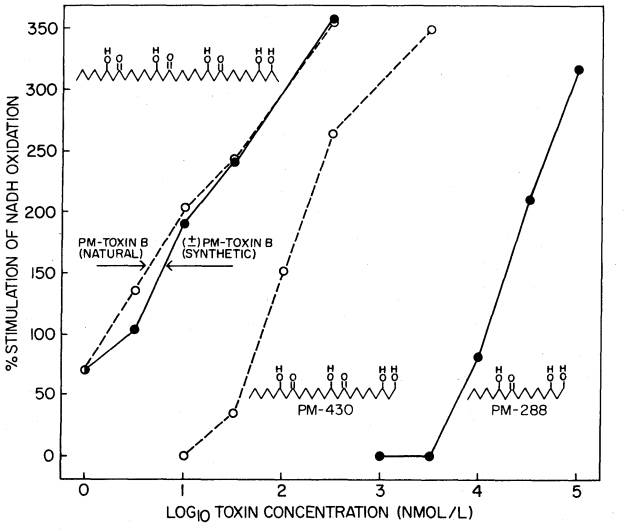


FIG. 5. Comparative Effects of Natural and Synthetic PM-Toxin B and Its Shorter Analogs on Oxidation of NADH by Mitochondria of Susceptible (W64AT) Corn.

for the maximum toxicity of PM-toxin analogs. Like the natural PM-toxins, the synthetics are all host-specific. None shows any activity on dark CO_2 fixation or NADH oxidation in resistant (W64AN) corn at 10 μM. Natural and synthetic PM-toxin B and PM-288 were also tested at 100 μM and were still inactive toward resistant corn at these high concentrations.

EXPERIMENTAL

NMR spectra were taken on JNM FX-90Q or GX-400 spectrometers. Low-resolution mass spectra (EI-MS) were obtained on a Hitachi RMU-6MG instrument. High-resolution mass spectra (HR-MS) and field-desorption mass spectra (FD-MS) were obtained on a Hitachi M-80 spectrometer. Infrared spectra (IR) were measured on a Shimadzu IR-435 spectrometer with films on NaCl plates or KBr disks.

Ethyl (±)-3-hydroxyoctanoate-3-methoxymethyl ether **2**. P_2O_5 (ca. 30 g) was added to a stirred solution of **1** (26.7 g, 142 mM) in dry $CHCl_3$ (150 ml) and methylal (150 ml) at room temperature. After 10 min., the mixture was decanted and the residue was washed out with EtOAc. The combined solution was washed successively with sat. $NaHCO_3$ and brine, and dried over $MgSO_4$. Evaporation of the solvent and subsequent distillation (74~80°C/0.25 mmHg) gave an oil of **1** (28.2 g, 85.6%). ^{1}H-NMR ($CDCl_3$): δ 0.89 (3H, br. t), 1.26 (3H, t, J=7 Hz), 2.60 and 2.42 (each 1H, ABX, J=16, 7, 6 Hz), 3.36 (3H, s), 3.98 (1H, quint, J=6 Hz), 4.14 (2H, q, J=7 Hz), 4.67 (2H, s). IR ν_{max}^{film} cm^{-1}: 1735, 1160, 1100, 1040.

(±)-3-Hydroxyoctanal-3-methoxymethyl ether **3** (*seg.* **a**). A mixture of **2** (28.2 g, 121 mM) in dry *n*-hexane (200 ml) was cooled to −78°C. DIBAL (121 ml of a 1 M solution in *n*-hexane, 121 mM) was added dropwise under N_2. The solution was stirred for 1 hr and dilute HCl and ether were added. The organic layer was separated, washed with water and dried ($MgSO_4$). Evaporation of the solvent, followed by distillation (70~74°C/0.6 mmHg) gave an oil of **3** (11.97 g, 54.8%). ^{1}H-NMR ($CDCl_3$): 0.89 (3H, br. t), 2.50 and 2.70 (each 1H, ABXY, J=16, 6, 5, 2 Hz), 3.35 (3H, s), 4.10 (1H, quint, J=7 Hz), 4.67 (2H, s), 9.80 (1H, t, J=2 Hz). IR ν_{max}^{film} cm^{-1}: 1725, 1150, 1100, 1040. EI-MS: m/z 157 ($M^+ - CH_3$, 14), 143 (28), 125 (50), 117 (65), 103 (65), 99 (50), 87 (100).

Methyl (±)-8-bromo-3-oxooctanoate **4**. The dianion was generated from methyl acetoacetate (23.2 g, 200 mM), 60% NaH (8.8 g, 220 mM) and *n*-BuLi (133 ml of 1.7 M solution in *n*-hexane, 212 mM) in dry THF (400 ml), as previously reported. 1,4-Dibromobutane (65.0 g, 300 mM) was added in one portion at 0°C. The mixture was stirred at 0°C for 10 min, and conc. HCl (40 ml), water (40 ml) and EtOAc (100 ml) were successively added. The organic phase was separated, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel, eluting with 20% EtOAc in *n*-hexane to give an oil of **4** (53.0 g, 52.5%). ^{1}H-NMR ($CDCl_3$): δ 1.88 (2H, quint, J=7 Hz), 2.57 (2H, t, J=7 Hz), 3.40 (2H, t, J=7 Hz), 3.45 (2H, s), 3.74 (3H, s). EI-MS: m/z 252 and 250 (M^+ +2 and M^+, 1), 221 and 219 (1), 179 and 177 (20 and 23), 171 (51), 129 (10), 116 (100).

(±)-8-Bromo-1,3-dihydroxyoctane-1,3-isopropylideneacetal **7** (*seg.* **b**). $NaBH_4$ (2.66 g, 70 mM) was added to a solution of **4** (53.0 g, 210 mM) in MeOH (800 ml) at 0°C. Stirring was continued for 1 hr and AcOH (18 ml) was added. The mixture was concentrated at room temperature. The residue was diluted with EtOAc and the solution was washed with sat. $NaHCO_3$ and brine, and dried over $MgSO_4$. Evaporation of the solvent gave a crude oil of **5** (51.5 g). ^{1}H-NMR ($CDCl_3$): δ 1.88 (2H, br. quint), 2.36 and 2.56 (each 1H, ABX, J=16, 7, 4 Hz), 2.90 (1H, d, J=2 Hz, OH), 3.41 (2H, t, J=7 Hz), 3.71 (3H, s), 4.00 (1H, m). EI-MS: m/z 236 and 234 ($M^+ + 2 - H_2O$ and $M^+ - H_2O$, 5), 103 (100), 74 (30).

DIBAL (520 ml of a 1 M solution in *n*-hexane, 520 mM) was added slowly to a cooled solution of **5** (*ca.* 51 g) in dry THF (500 ml) at −20°C. The mixture was stirred at −20°C for 1 hr and dilute HCl and Et_2O were added. The organic layer was separated, washed with sat. brine and dried ($MgSO_4$). Evaporation of the solvent gave a crude oil of **6** (*ca.* 50 g). ^{1}H-NMR ($CDCl_3$): δ 1.88 (2H, br. quint), 2.20 (2H, br. s, OH), 3.41 (2H, t, J=7 Hz), 3.86 (2H, t, J=6 Hz), *ca.* 3.9 (1H, m).

A solution of **6** (*ca.* 50 g) and *p*-TsOH (900 mg) in 2,2-dimethoxypropane and dry dioxane (100 ml each) was stirred at 20°C for 1 hr. Powdered $NaHCO_3$ was added and the solvent was evaporated. The residue was dissolved in EtOAc, and the solution was washed with sat. brine and dried with $MgSO_4$. Evaporation of the solvent, followed by distillation (85~90°C/0.2 mmHg) gave an oil of **7** (35.0 g, 63.1%). ^{1}H-NMR ($CDCl_3$): δ 1.38 and 1.44 (each 3H, s), 1.88 (2H, br. quint), 3.41 (2H, t, J=7 Hz), 3.6~4.2 (3H, m). IR ν_{max}^{film} cm^{-1}: 1380, 1370, 1250, 1100, 970. EI-MS: m/z 251 and 249 ($M^+ + 2 - CH_3$ and $M^+ - CH_3$, 70), 109 (100).

(±)-1-Bromo-6,8-dihydroxynonanes **9** *and* **10**. The dianion of 2,4-pentanedione (100 mM) was treated with 1.5 equiv. of 1,4-dibromobutane at 0°C for 5 min. and worked up as described above to give a crude oil of **8** (8.7 g). ^{1}H-NMR ($CDCl_3$): δ 2.07 (3H, s), 3.42 (3H, t, J=7 Hz), *ca.* 2.3 (2H, m), 5.52 (1H, s), and small peaks due to the keto-form [2.62 (br. t), 3.58 (s)].

The oil of **8** was treated with $NaBH_4$ (2.54 g, 67 mM) in MeOH (200 ml) at 10°C for 30 min and worked up as already described. Column chromatography on silica gel with 40% EtOAc in *n*-hexane gave an oil of **9** (2.58 g,

10.8%) and **10** (8.83 g, 37.0%).

9 (*syn*). ^{1}H-NMR ($CDCl_3$): δ 1.21 (3H, d, J=6 Hz), 3.0 (2H, br. s, OH), 3.42 (2H, t, J=7 Hz), 3.85 (1H, m), 4.04 (1H, m). IR ν_{max}^{film} cm^{-1}: 3350, 1460, 1435, 1370, 1125, 1080. EI-MS: *m/z* 207 and 205 (2), 181 and 179 (30), 163 and 161 (8), 97 (18), 89 (100), 81 (30), 71 (40), 55 (18), 45 (55).

10 (*anti*). ^{1}H-NMR ($CDCl_3$): δ 1.25 (3H, d, J=6 Hz), 2.6 (2H, br. s, OH), 3.42 (2H, t, J=7 Hz), 3.96 (1H, quint, J=5 Hz), 4.16 (1H, quint, J=6 Hz). IR ν_{max}^{film} cm^{-1}: 3350, 1460, 1435, 1370, 1090. EI-MS: *m/z* 207 and 205 (2), 181 and 179 (45), 163 and 161 (10), 97 (25), 89 (100), 81 (40), 71 (55), 55 (25), 45 (65).

(±)-1-Bromo-6,8-dihydroxynonane-6,8-isopropylideneacetals **11** (*seg.* **c**) *and* **12**. Acetonization of **9** (2.48 g, 10.4 mM) as above gave an oil of **11** (2.48, 85.8%), after purification by chromatography on silica gel with 5% EtOAc in *n*-hexane. Similarly, **12** (2.79 g, 84.7%) was also obtained.

11. ^{1}H-NMR ($CDCl_3$): δ 1.16 (3H, d, J=6 Hz), 1.40 and 1.43 (each 3H, s), 3.6~4.2 (2H, m), 3.41 (2H, t, J=7 Hz). IR ν_{max}^{film} cm^{-1} 1380, 1260, 1200, 1120. EI-MS: *m/z* 265 and 263 (M^++2−CH_3 and M^+−CH_3, 15), 205 and 203 (3), 163 and 161 (2), 123 (15), 59 (18).

12. 1H-NMR ($CDCl_3$): δ 1.18 (3H, d, J=6 Hz), 1.35 (6H, s), 3.41 (2H, t, J=7 Hz), 3.6~4.2 (2H, m). IR ν_{max}^{film} cm^{-1}: 1380, 1220, 1180, 1130, 1000. EI-MS: *m/z* 265 and 263 (M^++2−CH_3 and M^+−CH_3, 23), 205 and 203 (4), 163 and 161 (4), 123 (20), 59 (25).

(±)-1-Bromo-6,8-dihydroxynonane-6,8-benzylideneacetals **13** *and* **14**. A mixture of **9** (170 mg, 0.72 mM) and *p*-TsOH (12.5 mg) in benzaldehyde dimethylacetal (2 ml) and dry dioxane (2 ml) was stirred at room temperature for 1 hr. Powdered $NaHCO_3$ was added and the solvent was evaporated. Preparative TLC on silica gel with 15% EtOAc in *n*-hexane gave an oil of **13** (198 mg, 85.0%). Similarly, **14** (530 mg, 80.0%) was also obtained.

13. ^{1}H-NMR (C_6D_6, 400 MHz): δ 1.04 (1H, dt, J=12.9 and 2.5 Hz), 1.18 (3H, d, J=6.1 Hz), 3.40 (1H, m), 3.56 (1H, ddq, J=10.9, 6.1, 2.5 Hz), 5.43 (1H, s). EI-MS: *m/z* 328 and 326 (M^++2 and M^+, 44 and 40), 177 (16), 123 (20), 107 (55), 105 (100).

14 (**14A** and **14B**). ^{1}H-NMR (C_6D_6, 400 MHz): δ 1.17 (1.5H, d, J=6.8 Hz), 3.67 (0.5H, m), 4.25 (0.5H, dq, J=6.8 Hz), 5.79 (0.5H, s) in **14A** and 1.15 (1.5H, d, J=6.1 Hz), 3.78 (0.5H, m), 3.93 (0.5 Hz, m), 5.68 (0.5H, s) in **14B**.

A mixture of **9** (10 mg), Ac_2O (1 ml), and dry pyridine (2 ml) was treated at 0°C for 18 hr and the solvent was evaporated at room temperature to give the *syn*-diacetate. Similarly, the *anti*-diacetate was also obtained.

syn-Diacetate of **9**. ^{1}H-NMR ($CDCl_3$): δ 1.24 (3H, d, J=6 Hz), 2.03 and 2.05 (each 3H, s), 3.40 (2H, t, J=7 Hz), 4.93 (2H, m). ^{13}C-NMR ($CDCl_3$) δ 20.1 (q), 21.1 (q), 21.2 (q), 24.3 (t), 28.0 (t), 32.6 (t), 33.3 (t), 34.1 (t) 40.3 (t), 68.1 (d), 71.1 (d), 170.5 (s)×2. IR ν_{max}^{film} cm^{-1}: 1735, 1370, 1240, 1020.

anti-Diacetate of **10**. ^{1}H-NMR ($CDCl_3$): δ 1.22 (3H, d, J=6 Hz), 2.01 and 2.02 (each 3H, s), 3.40 (2H, t, J=7 Hz), 4.96 (2H, br. quint). ^{13}C-NMR ($CDCl_3$): δ 20.4 (q), 21.0 (q), 21.1 (q), 24.3 (t), 28.0 (t), 32.6 (t), 33.3 (t), 34.6 (t), 40.5 (t), 67.1 (d), 70.3 (d), 170.5 (s)×2. IR ν_{max}^{film} cm^{-1}: 1735, 1370, 1240, 1140, 1020.

1,3,11-Trihydroxy-9-oxohexadecane-1,3-isopropylideneacetal-11-methoxymethyl ether **16**. A mixture of Mg turnings (1.92 g), **7** (19.1 g, 72 mM) and a crystal of I_2 in dry THF (70 ml) was refluxed under N_2 for 20 min. A solution of **3** (12.2 g, 65 mM) in dry THF (60 ml) was added dropwide at −10°C for 10 min. The mixture was warmed to room temperature during 45 min, and poured into cooled sat. NH_4Cl solution. The mixture was worked up to give a crude oil of **15** (22.83 g). A mixture of **15** (22.83 g) and PDC (46 g, 122 mM) in dry DMF (100 ml) was stirred at room temperature for 18 hr. The mixture was diluted with water (800 ml) and ether. The ether was washed with water and dried over $MgSO_4$. Evaporation of the solvent and subsequent column chromatography on silica gel with 20% EtOAc in *n*-hexane gave an oil of **16** (15.8 g, 69.6%). ^{1}H-NMR ($CDCl_3$): δ 0.89 (3H, br. t), 1.38 and 1.44 (each 3H, s), 2.44 (2H, t, J=7 Hz), 2.44 and 2.74 (each 1H, ABX, J=16, 7, 5 Hz), 3.33 (3H, s), 3.6~4.2 (4H, m), 4.63 (2H, s). IR ν_{max}^{film} cm^{-1}: 1712, 1200, 1150, 1100, 1040. EI-MS: *m/z* 357 (M^+−CH_3, 50), 295 (100). HR-MS Found: M^+−CH_3 *m/z* 357.2611; Calc. for $C_{20}H_{37}O_5$: 357.2639.

1,3,11-Trihydroxy-9-oxohexadecane-9-ethylenedithioacetal **17**. BF_3·etherate (3.37 ml) was added to a solution of **16** (15.8 g, 42.5 mM) and ethanedithiol (16 g, 170 mM) in dry CH_2Cl_2 (180 ml). The solution was stirred at room temperature for 18 hr and 5% NaOH (100 ml) was added. The organic layer was separated, washed with brine and dried ($MgSO_4$). Evaporation of the solvent yielded the crude product, which was chromatographed on silica gel, eluting with 3% MeOH in CH_2Cl_2, to afford an oil of **17** (9.73 g, 62.8%). ^{1}H-NMR ($CDCl_3$): δ 0.89 (3H, br. t), 2.01 (2H, d, J=5 Hz), 2.40 (2H, br. s, OH), 3.32 (4H, s), 3.42 (1H, d, J=2 Hz, OH), 3.7~4.1 (4H, m). IR ν_{max}^{film} cm^{-1}: 3400, 1640, 1460, 1420, 1050. EI-MS: *m/z* 364 (M^+, 10), 301 (8), 249 (45), 219 (95), 119 (100). HR-MS Found: M^+ *m/z* 364.2146; Calc. for $C_{18}H_{36}O_3S_2$: 364.2104.

1,3,11-Trihydroxy-9-oxohexadecane-9-ethylenedithioacetal-1-trityl ether **18**. A mixture of **17** (9.60 g, 26.3 mM), trityl chloride (8.07 g, 29.0 mM), DMAP (161 mg, 1.31 mM) and triethylamine (6.6 ml) in dry CH_2Cl_2 (25 ml) was stirred under N_2 at room temperature for 18 hr. The mixture was diluted with CH_2Cl_2 and the organic phase was washed with sat. NH_4Cl and brine, dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel, eluting with CH_2Cl_2, to afford an oil of **18** (15.48 g, 96.9%). ^{1}H-NMR

($CDCl_3$): δ0.89 (3H, br. t), 2.00 (2H, d, J=5 Hz), 2.88 (1H, d, J=2 Hz, OH), 3.30 (4H, s), 3.2~3.5 (2H, m), 3.44 (1H, d, J=2 Hz, OH), 3.76 (1H, br. s), 4.00 (1H, br. s), 7.1~7.6 (15H, m). IR ν_{max}^{film} cm^{-1}: 3450, 1595, 1490, 1450, 1220, 1070. EI-MS: m/z 364 (M^+ − trityl, 5), 345 (7), 302, (15), 249 (45), 243 (40), 219 (95), 119 (100).

3,11-Di(benzyl)oxy-1-hydroxy-9-oxohexadecane-9-ethylenedithioacetal **20**. A mixture of **18** (15.34 g, 25.2 mM) and 60% NaH (4.04 g, 100 mM) in dry THF (100 ml) was stirred at room temperature for 1.5 hr under N_2, and then Bu_4NI (270 mg, 1 mM) and benzyl bromide (17.2 g, 100 mM) were added. The mixture was stirred for 2 days and diluted with EtOAc, and the organic layer was washed with sat. NH_4Cl and brine, dried over $MgSO_4$, and evaporated to afford a crude oil of **19**. This crude oil was dissolved in MeOH–$CHCl_3$ (1:1, 250 ml) and stirred with *p*-TsOH (430 mg, 2.52 mM) at 20°C for 2.5 hr. Powdered $NaHCO_3$ was added, the solvent was removed and the residue was dissolved in EtOAc. The organic phase was washed with brine and dried with $MgSO_4$. Evaporation of the solvent, followed by column chromatography on silica gel with 20% EtOAc in *n*-hexane gave a pale yellow oil of **20** (5.45 g, 39.9%).

19. ^{1}H-NMR ($CDCl_3$): δ0.89 (3H, br. s), 2.08 and 2.32 (each 1H, ABX, J=15, 7, 4 Hz), 3.20 (2H, t, J=6 Hz), 3.23 (4H, s), 3.60 (2H, br. s), 4.44 and 4.56 (each 1H, ABq, J=11 Hz), 4.32 and 4.44 (each 1H, ABq, J=11 Hz). IR ν_{max}^{film} cm^{-1}: 1595, 1490, 1450, 1070. EI-MS: m/z 786 (M^+, 0.1), 544 (M^+ − trityl, 2), 261 (30), 243 (90), 197 (35), 183 (85), 105 (80), 91 (100).

20. ^{1}H-NMR ($CDCl_3$): δ0.89 (3H, br. t), 2.08 and 2.32 (each 1H, ABX, J=15, 7, 4 Hz), 2.3 (1H, br. s, OH), 3.27 (4H, s), 3.44~3.84 (4H, m), 4.44 and 4.56 (each 1H, ABq, J=11 Hz), 4.44 and 4.60 (each 1H, ABq, J=11 Hz). IR ν_{max}^{film} cm^{-1}: 3430, 1495, 1455, 1350, 1090, 1060. EI-MS: m/z 544 (M^+, 2), 483 (1), 453 (3), 438 (12), 347 (18), 339 (16), 203 (25), 191 (22), 151 (15), 91 (100). HR-MS Found: M^+ 544.3018; Calc. for $C_{32}H_{48}O_3S_2$: 544.3041.

3,11-Di(benzyl)oxy-9-oxohexadecanal-9-ethylenedithioacetal **21**. A solution of **20** (10.43 g, 19.2 mM) in dry pyridine (100 ml) was added to a slurry of CrO_3 (9.6 g, 96 mM) in dry pyridine (500 ml). The mixture was stirred at room temperature for 18 hr. Ether (430 ml) was added, the mixture filtered, and the residue washed out with ether. The ether extract was washed with dilute HCl, 5% NaOH and sat. brine, and dried ($MgSO_4$). Evaporation of the solvent, followed by chromatography on silica gel with 20% EtOAc in *n*-hexane gave an oil of **21** (5.26 g, 50.5%). ^{1}H-NMR ($CDCl_3$): δ0.89 (3H, br. t), 2.08 and 2.32 (each 1H, ABX, J=15, 7, 4 Hz), 2.6 (2H, m), 3.26 (4H, s), 3.60 (1H, m), 3.88 (1H, br. quint, J=5 Hz), 4.44 and 4.56 (each 1H, ABq, J=11 Hz), 4.51 (2H, s), 7.1~7.7 (10H, s), 9.76 (1H, t, J=2 Hz). IR ν_{max}^{film} cm^{-1}: 2700, 1720, 1495, 1455, 1350, 1090, 1060. EI-MS: m/z 542 (M^+, 0.2), 524 ($M^+ - H_2O$, 0.2), 481 (2), 337 (12), 329 (15), 309 (5), 267 (12), 229 (45), 203 (40), 191 (20), 91 (100).

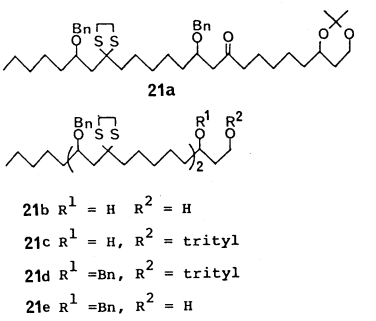


FIG. 6.

6,14,22-Tri(benzyl)oxy-30,32-dihydroxy-8,16,24-trioxotritriacontane-8,16-di(ethylenedithio)-acetal-30,32-isopropyrideneacetal **23**. Conversion of C_{15}-aldehyde **21** to C_{33}-acetonide **23** *via* C_{24}-aldehyde **22** was carried out in almost the same manner as described for **15** to **21**.

21a (oil). ^{1}H-NMR ($CDCl_3$): δ0.89 (3H, br. t), 1.38 and 1.43 (each 3H, s), 2.42 (2H, t, J=7 Hz), 2.46 and 2.72 (each 1H, ABX, J=16, 7, 5 Hz), 2.08 and 2.32 (each 1H, ABX, J=15, 7, 4 Hz), 3.26 (4H, s), 3.60 (1H, m), 3.90 (4H, m), 4.47 (2H, s), 4.44 and 4.56 (each 1H, ABq, J=11 Hz), 7.1~7.6 (10H, m). IR ν_{max}^{film} cm^{-1}: 1710, 1495, 1455, 1380 1370, 1200, 1100, 1065. EI-MS: m/z 726 (M^+, 0.1), 712 ($M^+ - CH_3$, 2), 603 (25), 549 (30), 453 (60), 355 (75), 309 (40), 203 (100), 191 (100). HR-MS Found: M^+ m/z 726.4387; Calc. for $C_{43}H_{66}O_5S_2$: 726.4348.

21b (oil). ^{1}H-NMR ($CDCl_3$): δ0.89 (3H, br. t), 1.96~2.46 (4H, m), 3.26 (8H, s), 3.62 (2H, m), 3.90 (3H, m), 4.50 (4H, m), 7.2~7.5 (10H, m). FD-MS: m/z 762 (M^+, 100).

21c (oil). ^{1}H-NMR ($CDCl_3$): δ0.89 (3H, br. t), 3.3 (2H, m), 3.25 (8H, s), 3.6 (2H, m), 3.76 (1H, m), 4.48 (4H, m), 7.3 (25H, m). IR ν_{max}^{film} cm^{-1}: 3470, 1595, 1490, 1445, 1065. FD-MS: m/z 1002 ($M^+ - 2H$, 100).

21d (oil). ^{1}H-NMR ($CDCl_3$): δ0.89 (3H, br. t), 3.20 (2H, t, J=6 Hz), 3.25 (8H, s), 3.56 (3H, m), 4.32 and 4.44 (each 1H, ABq, J=11 Hz), 4.44 and 4.56 (each 2H, ABq, J=11 Hz). IR ν_{max}^{film} cm^{-1}: 1595, 1490, 1450, 1065.

21e (oil). ^{1}H-NMR ($CDCl_3$): δ0.89 (3H, br. t), 2.08 and 2.32 (each 2H, ABX, J=15, 7, 4 Hz), 3.26 (8H, s), 3.4~3.9 (5H, m), 4.44 and 4.56 (each 2H, ABq, J=11 Hz), 4.44 and 4.60 (each 1H, ABq, J=11 Hz), 7.31 (15H, m). IR ν_{max}^{film} cm^{-1}: 3430, 1600, 1495, 1350, 1090, 1060. EI-MS: m/z 852 (M^+, 1), 759 (10), 745 (15), 655 (45), 647 (30), 339 (100), 303 (30). HR-MS Found: M^+ m/z 852.4350; Calc. for $C_{49}H_{72}O_4S_4$: 852.4310.

22 (oil). ^{1}H-NMR ($CDCl_3$): δ0.89 (br. t), 2.08 and 2.32 (each 2H, ABX, J=15, 7, 4 Hz), 2.6 (2H, m), 3.26 (8H, s), 3.56 (2H, m), 3.86 (1H, m), 4.51 (6H, m). IR ν_{max}^{film} cm^{-1}: 2700, 1720, 1495, 1455, 1350, 1090, 1060. FD-MS: m/z 850

(M^+, 50), 831 (100).

23 (oil). ^{1}H-NMR ($CDCl_3$): 0.89 (3H, br. t), 1.15 (3H, d, J=6 Hz), 1.39 and 1.42 (each 3H, s), 2.42 (2H, t, J=7 Hz), 2.08 and 2.32 (each 2H, ABX, 15, 7, 4 Hz), 2.46 and 2.72 (each 1H, ABX, J=16, 7, 5 Hz), 3.25 (8H, s), 3.6~3.9 (5H, m), 4.47 (6H, m). IR ν_{max}^{film} cm^{-1}: 1712, 1495, 1455, 1380, 1200, 1180, 1110, 1090, 1065. FD-MS: m/z 1048 (M^+, 100).

*6,14,22-Tri(benzyl)oxy-30,32-dihydroxy-8,16,24-*trioxotritriacontane **25**. A mixture of **23** (820 mg, 0.78 mM) in AcOH (35 ml) water (15 ml), and THF (20 ml) was stirred at 50°C for 5 hr. The solvent was evaporated at room temperature to give an oil of **24** (780 mg, 97.7%). A solution of **24** (760 mg, 0.76 mM), $HgCl_2$ (1.14 g) and HgO (350 mg) in CH_3CN (25 ml) and water (1.5 ml) was stirred at 25°C for 4 hr under N_2. The mixture was filtered and the filtrate was diluted with water and $CHCl_3$. The organic phase was separated, washed with sat. NH_4Cl solution and dried ($MgSO_4$). Evaporation of the solvent, followed by column chromatography on silica gel with 2% i-PrOH in CH_2Cl_2 afforded an oil of **25** (430 mg, 64.9%).

24. ^{1}H-NMR ($CDCl_3$): δ 0.89 (3H, br. t), 1.19 (3H, d, J=6 Hz), 3.26 (8H, s), 3.6~3.9 (5H, m), 4.49 (6H, m). IR ν_{max}^{film} cm^{-1}: 3400, 1709, 1410, 1495, 1455, 1090, 1060. FD-MS: m/z 1008 (M^+, 100).

25. ^{1}H-NMR ($CDCl_3$): δ 0.88 (3H, br. t), 1.19 (3H, d, J=6 Hz), 2.41 (6H, t, J=6 Hz), 2.41 and 2.74 (each 3H, ABX, J=16, 7, 5 Hz), 2.96 (2H, br. s, OH), 3.92 (5H, m), 4.48 (6H, s), 7.29 (15H, s). IR ν_{max}^{film} cm^{-1}: 3400, 1710, 1495, 1550, 1090, 1060. FD-MS: m/z 856 (M^+, 100).

6,14,22,30,32-Pentahydroxy-8,16,24-trioxotritriacontane (*PM-toxin B*). A mixture of **25** (50 mg) and activated Pd black (200 mg) in AcOH–dioxane–water (4:1:1, 35 ml) was treated with 1 atom/cm^2 of H_2 at 35°C for 24 hr. The mixture was filtered and the filtrate concentrated at room temperature. The residue was again treated with H_2 on activated Pd black (160 mg) in the same solvent mixture (35 ml) at 35°C for 24 hr. The mixture was filtered and the filtrate was concentrated. Preparative TLC of the oily residue on silica gel with 10% MeOH in $CHCl_3$, followed by precipitation from MeOH gave a colorless powder of (±)-PM-toxin B (12.7 mg, 37.6%). ^{1}H-NMR (400 MHz, d_5-pyridine, Fig. 4): δ 0.83 (3H, t, J=7.0 Hz), 1.39 (3H, d, J=6.1 Hz), 1.90 (1H, dt, J=13.9, 9.0 Hz), 2.56 (2H, t, J=7.0 Hz), 2.57 (4H, t, J=7.0 Hz), 2.62, 2.63, and 2.65 (each 1H, dd, J=15.1, 3.9 Hz), 2.81, 2.82, and 2.83 (each 1H, dd, J=15.1, 8.7 Hz), 4.09 (1H, m), 4.36 (1H, m), 4.43 (3H, m). ^{13}C-NMR (22.5 MHz, d_5-pyridine, Fig. 5): δ 14.1 (q), 22.9 (t), 23.9 (t) × 3, 24.7 (q), 26.1 (t) × 2, 29.1 (t), 29.5 (t) × 3, 32.1 (t), 38.2 (t) × 3, 38.7 (t), 43.8 (t) × 3, 46.5 (t), 51.3 (t) × 3, 67.7 (d) × 3, 71.4 (d), 210.3 (s) × 3. IR ν_{max}^{KBr} cm^{-1}: 3350, 2930, 2850, 1703, 1460, 1400, 1385, 1110, 1090. The IR spectrum of synthetic PM-toxin B was slightly different from that of the natural one, especially at the C=O bands; ν1709 (natural) and 1703 (synthetic). This may be the effects of the unnatural stereoisomers differing with that of natural PM-toxin B. FD-MS: m/z 609 (M^++Na, 100), 591 ($M^+ - H_2O$+Na, 14), 509 (28), 486 (10), 407 (27), 384 (7), 367 (78), 344 (14).

6,14,22,24-Tetrahydroxy-8,16-dioxotetracosane (*PM-430*) *and 6,14.16-Trihydroxy-8-oxohexadecane* (*PM-288*). As described above, PM-430 was prepared *via* dethioacetalization of **21b** followed by debenzylation as a colorless powder; precipitation was from *n*-hexane. Similarly, dethioacetalization of **17** gave a colorless powder of PM-288; precipitation was from *n*-hexane.

PM-430. ^{1}H-NMR (400 MHz, d_5-pyridine): δ 0.83 (3H, t, J=7.0 Hz), 2.01 (2H, m), 2.55 and 2.57 (each 2H, t, J=7.0 Hz), 2.62 and 2.65 (each 1H, dd, J=15.1, 3.9 Hz), 2.80 and 2.83 (each 1H, dd, J=15.1, 9.0 Hz), 4.20 (3H, m), 4.45 (2H, m), ^{13}C-NMR (22.5 MHz, d_5-pyridine): δ13.9 (q), 22.7 (t) × 2, 23.9 (t), 25.5 (t), 25.7 (t), 25.8 (t), 29.4 (t), 29.5 (t), 31.9 (t), 37.9 (t), 38.0 (t), 38.3 (t), 40.7 (t), 43.7 (t) × 2. 50.9 (t) × 2, 60.4 (t), 67.7 (d) × 2, 69.9 (d), 210.1 (s) × 2. IR ν_{max}^{KBr} cm^{-1}: 3350, 2930, 2850, 1703, 1460, 1400, 1385, 1110. FD-MS: m/z 453 (M^++Na, 100), 883 ($2M^+$+Na, 95), 431 (M^++H, 23).

PM-288. ^{1}H-NMR (400 MHz, d_5-pyridine): δ 0.83 (3H, t, J=7.0 Hz), 2.01 (2H, m), 2.56 (2H, t, J=7.0 Hz), 2.64 (1H, dd, J=15.1, 3.9 Hz), 2.82 (1H, dd, J=15.1, 8.7 Hz), 4.20 (3H, m), 4.45 (1H, m). ^{13}C-NMR (22.5 MHz, d_5-pyridine): δ14.0 (q), 22.8 (t), 23.9 (t), 25.6 (t), 26.0 (t), 29.5 (t), 32.0 (t), 38.1 (t), 38.4 (t), 40.9 (t), 43.7 (t), 51.0 (t), 60.2 (t), 67.7 (d), 69.5 (d), 210.2 (s). IR ν_{max}^{KBr}: 3350, 2930, 2850, 1703, 1460, 1400, 1385, 1110, 1055, FD-MS: m/z 903 ($3M^+$+K, 10), 887 (M^++Na, 79), 615 ($2M^+$+K, 16), 599 ($2M^+$+Na, 100), 577 ($2M^+$+H, 16), 311 (M^++Na, 10), 289 (M^++H, 16).

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