Synthesis and Biological Activity of a New Type of PM-Toxin Analogue Containing Oxygen Atoms in the Carbon-Chain Skeleton

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Received September 5, 1988

PM-toxins are host-specific pathotoxins produced by the corn fungal pathogen *Phyllosticta maydis*. Three PM-toxin analogues of a new type which have oxygen atoms in the skeletal carbon chain were synthesized as stereoisomeric mixtures. The linear ester PML-777 was about 100-times less active than the potent synthetic analogue PM-777 in an NADH oxidation assay using corn mitochondria. A large cyclic analogue, PMC₃₂-7777, was only about 4- to 10-times less active than the parent linear ester PML-777, but over 500-times more active than a smaller sized analogue, PMC₂₀-711. The striking loss of biological activity in PML-777 clearly shows the significance of a carbon-chain skeleton like PM-777 for higher biological activity. The unexpectedly slight decrease of activity from PML-777 to PMC₃₂-7777 may suggest that the active conformation of the linear analogue (PML-777) was similar to that of the cyclic analogue (PMC₃₂-7777).

PM-toxins (A to D),^{1~3)} the host-specific pathotoxins which selectively damage only corn with Texas male-sterile cytoplasm (Tms-corn), were previously isolated from the fungal corn pathogen *Phyllosticta maydis*. Interestingly, each related component showed biological activity almost identical to that observed for HMT-toxins^{4~6)} isolated from the taxonomically distinguished corn fungal pathogen *Helminthosporium maydis* (race T), which also damages only Tms-corn. We are interested in understanding the structure-activity relationships and the modes of action of these toxins. Various analogues of HMT-toxin and PM-toxin, including a stereoisomeric mixture of PM-toxin B, were previously synthesized,^{7~9)} and the results clarified some structural requirements for the higher host-specific toxicity. One of the prominent factors required for potent activity was molecular flexibility of the skeletal carbon chain to make

involved conformations. Namely, an increase of the rigidity by introducing either a shorter or conjugated diene spacer like PM-373, -575, -595, PM-777t or PM-B' caused a marked decrease of activity (Fig. 1), while the carbon asymmetry and the orientation and spacing of oxygenic groups in the carbon straight-chain had little or no significant influence on the activity.

Based on these results, we attempted to synthesize a new type of PM-toxin analogue, PML-777, PMC₃₂-7777 and PMC₂₀-711, all of which contained ester linkages in the long straight carbon-chain skeleton (Fig. 2), in order to clarify the active conformation of PM-toxins. PML-777 is a linear bisester analogue; the other two are cyclic analogues related to PML-777, PMC₃₂-7777 being a large-sized cyclic analogue corresponding to a ring form of PML-777, and PMC₂₀-711 being a medium-sized analogue. The linear type of analogue

Fig. 1. Structure of Some Potent Synthetic Analogues and Their Relative Activity on NADH Oxidation in Mitochondria.

*Relative activity (%) when the activity of native PM-toxins was set at 100%.

Fig. 2. Structure of Native PM-Toxins A and B and New Synthetic Analogues.

PML-777 was especially expected to retain higher toxic activity because the orientation, spacing of oxygenic groups and the chain length are the same as those of PM-777, which possesses potent biological activity.

This paper describes the syntheses and biological activity of PML-777 and two of its cyclic analogues, $\text{PMC}_{32}\text{-7777}$ and $\text{PMC}_{20}\text{-711}$ (Fig. 2).

Results and Discussion

The synthetic outlines of PML-777, $\text{PMC}_{32}\text{-7777}$

and $\text{PMC}_{20}\text{-711}$ are shown in Figs. 3 and 4. Intermolecular condensation between fragments A and C led to the corresponding linear analogue, PML-777, while intramolecular condensation between fragments A and B (or 1,3-propanediol) led to the corresponding cyclic analogues, $\text{PMC}_{32}\text{-7777}$ and $\text{PMC}_{20}\text{-711}$, respectively. All the analogues were stereoisomeric mixtures.

The preparation of fragments A and B starting from bis- β -oxoester **1**¹⁰⁾ is shown in Fig. 3. Treatment of **1** with 1,2-ethanedithiol (3 equiv.) and boron trifluoride-etherate in di-

Fig. 3. Synthetic Scheme for Fragments A and B (8 and 9) Leading to PML-777, PMC₃₂-7777 and PMC₂₀-711.

Fig. 4. Synthetic Scheme for PML-777, PMC₃₂-7777 and PMC₂₀-711.

chloromethane at room temperature afforded bisthioketal 2 in a 76% yield. Reduction of 2 with an excess of lithium aluminum hydride and subsequent Swern's oxidation¹¹⁾ of the resulting diol gave the corresponding dialdehyde 3 in an 89% yield. Condensation of the enolate anion of *tert*-butyl acetate (2 equiv.) with 3 gave the bisaldol adduct 5 (*c.* 100%). After protecting the hydroxyl functions of 5 as its THP ether by treating with an excess of dihydropyran and *p*-toluenesulfonic acid in dioxane, reduction of the *tert*-butyl ester 7 with lithium aluminum hydride gave the desired fragment B (9) in an almost quantitative yield. Fragment A (8) was obtained from the corresponding isopropyl acetate adduct 4 in a 77% yield, *via* saponification of the ethyl ester

of the THP derivative 6 with sodium hydroxide in aqueous methanol because conversion of the *tert*-butyl adduct 7 to fragment A by saponification failed.

The preparation of PML-777 and the two cyclic analogues is shown in Fig. 4. Esterification or lactonization between fragment A (8) and each alcohol fragment (9, 10⁸⁾ and 1,3-propanediol) was accomplished by treating with equimolar amounts of dicyclohexylcarbodiimide (DCC) in the presence of dimethylaminopyridine¹²⁾ (0.1 equiv.) as a catalyst at room temperature. Lactones 13 and 15 could not be obtained in analytically pure forms and, therefore, each conjugate was treated with warm aqueous acetic acid containing tetrahydrofuran or refluxed with dilute hy-

Table I. ^{13}C -NMR DATA OF SYNTHETIC ANALOGUES
(22.5 MHz, CDCl_3 , ppm)

	PM-777	PML-777	PMC ₃₂ -7777	PMC ₂₀ -711
CH ₂	28.2	28.2	28.0	27.2
CH ₂	22.3*	22.4*	22.8	22.6
CH ₂	43.0***	43.0	42.8	42.2, 42.3
C=O	N.D.	210.2	210.4	210.8
CH ₂	48.8	48.1**	48.1*	48.0, 48.1
CHOH	67.3**	64.3	64.5**	64.4, 64.5
CH ₂	35.9****	40.9***	41.0	40.9
CH ₂ C=O C=O C=O	24.9	171.2	171.5	171.5
CH ₂ O O O	28.7			
CH ₂ CH ₂ CH ₂ CH ₂	23.2	59.1	61.2	61.8, 62.1
CH ₂ CH ₂ CH ₂ CH ₂	43.3***	40.7***	35.2	27.7
C=O C=O CHOH	N.D.	208.4	64.8**	
CH ₂ CH ₂ CH ₂	48.8	49.5**	48.8*	
CHOH CHOH C=O	67.5**	67.7	211.2	
CH ₂ CH ₂ CH ₂	36.3****	36.4	42.8	
CH ₂ CH ₂ CH ₂	24.9	24.9	22.8	
CH ₂ CH ₂ CH ₂	31.5	31.5	28.0	
CH ₂ CH ₂	22.9*	22.9*		
CH ₃ CH ₃	13.8	13.8		

* Assignments are exchangeable.

drochloric acid in ethanol to afford the corresponding hydroxyl compound (**12**, **14** and **16**). The yields were 43 to 7%, respectively. Reaction of the thioketals (**12**, **14** and **16**) with mercuric chloride in aqueous acetonitrile at room temperature gave the desired PM-toxin analogues (42 to 62% yields), respectively.

The structures of all the analogues were confirmed by means of their MS data and elemental analyses, ^{13}C - (Table I) and ^1H -NMR spectra, and IR spectra described in the experimental section.

The relative biological activities of the three newly synthesized analogues (PML-777, PMC₃₂-7777 and PMC₂₀-711) and of PM-777 were evaluated for their stimulation of NADH oxidation in isolated Tms-corn mitochondria,¹³⁾ and compared by estimating the concentration required to effect 50% of each maximum activity (ED_{50} , Fig. 5). PM-777 is a synthetic PM-toxin analogue composed of a carbon-chain skeleton and with

activities comparable to those of natural PM-toxins.

Although the orientation and spacing of the oxygenic groups of PML-777 were the same as those of PM-777, the analogue PML-777 was, unexpectedly, about 100-times less active than PM-777. This clearly shows that the introduction of an ester linkage into the carbon-chain skeleton of the toxin molecule caused a drastic loss of activity. Therefore, we conclude that the carbon-carbon chain skeleton of the PM-toxin molecule was an important factor for maximum activity. The low activity of PML-777 relative to PM-777 cannot be clearly explained at the present stage; however, it is possible that disturbance of the formation of optimal conformations for maximum activity was one of the major factors. When the relative activity between PML-777 and PM-777 was compared in detail, the activity of PML-777 at a higher concentration of around 10^{-7} M appeared that of PM-777. Perhaps detoxifi-

Fig. 5. Comparative Effects of Synthetic PM-Toxin Analogues on the Oxidation of NADH by Mitochondria Isolated from Susceptible W64A-Tms Corn.

ΔPM-777; ○ PML-777; ● PMC₃₂-7777; ▲ PMC₂₀-711.

cation due to immediate hydrolytic-cleavage of the ester linkages in the mitochondria occurred and, therefore, the activity of PML-777 at the lower concentration of around 10^{-8} M was strikingly reduced.

The cyclic analogue PMC₃₂-7777 was 1000-times less active than PM-777, but only about 4- to 10-times less active than the corresponding linear analogue PML-777. Since PMC₃₂-7777 can be regarded as one of the possible conformations of the linear PML-777, a small decrease in the activity of PMC₃₂-7777 relative to PML-777 might be primarily due to an increase of molecular rigidity by ring formation. Thus, the biological activity of PMC₃₂-7777 was unexpectedly high. Furthermore, PMC₃₂-7777, which was considered to retain higher molecular rigidity than some less potent linear analogues (*e.g.*, PM-373 and -575 and PM-777t⁸⁾) by having either a shorter or conjugated diene spacer, showed virtually identical activity to the linear analogues (Fig. 1). In fact, another cyclic analogue (PMC₂₀-711) that is smaller than PMC₃₂-7777 showed drastically decreased activity (over 100-times less than PMC₃₂-7777) and was over 10-times less active than the corresponding

linear analogue PM-7 (PM-288).⁷⁾ These results suggest that the active conformation of the linear analogue PML-777 may resemble that of the cyclic analogue PMC₃₂-7777, but not PMC₂₀-711.

These synthetic analogues exhibited specificity because they had no significant effect on an NADH oxidation assay in resistant corn mitochondria with normal cytoplasm even at high concentrations (data not shown).

In summary, we prepared three new PM-toxin analogues containing oxygen atoms in the carbon-chain skeleton and clarified that this skeleton was an important structure for the maximum biological activity of PM-toxins. We also found that even the cyclic analogue PMC₃₂-7777 retained considerable activity. The finding of bioactive PML-777 might be helpful in investigating active conformations of the PM-toxin. When the chiral forms of both PM-777 and PML-777 are synthesized, a comparison of each conformational structure determined by means of an X-ray analysis might reveal the most preferable conformation for maximum biological activity.

A synthesis of both chiral analogues is currently in progress.

Experimental

Infrared spectra (IR) were obtained using a Shimadzu IR-435 spectrometer with films on NaCl plates or with KBr disks. The NMR data were obtained using JEOL JNM FX-90Q and FX-400 spectrometers. High-resolution mass spectra (HR-MS) and field-desorption mass spectra (FD-MS) were obtained on a Hitachi M-80 spectrometer.

Dimethyl, 3,9-dioxoundacadioate-3,9-di(ethylenedithio)-ketal (2). Boron trifluoride etherate (15 ml) was added to a solution of bisoxoester **1**⁽¹⁰⁾ (42.5 g, 156 mm) and 1,2-ethanedithiol (32.4 g, 343 mm) in dry dichloromethane (400 ml). After 18 hr of stirring at room temperature, the mixture was washed with water and concentrated to afford a yellow solid. Crystallization from MeOH gave 50.6 g (76%) of **2**, mp 71.5–72.0°C. NMR δ_{H} (90 MHz, CDCl_3): *c.* 1.5 (6H, m), *c.* 2.1 (4H, m), 3.04 (4H, s), 3.29 (8H, s), 3.70 (6H, s). IR ν_{max} (KBr) cm^{-1} : 1735. HR-MS m/z Found: M^+ 424.0817. Calcd. for $\text{C}_{17}\text{H}_{28}\text{O}_4\text{S}_4$: 424.0868.

3,9-Dioxoundacadiol-3,9-di(ethylenedithio)ketal (3). Thioketal **2** (42.4 g, 100 mm) in dry tetrahydrofuran (THF, 100 ml) was slowly added to a slurry of LiAlH_4 (8 g, 210 mm) in dry THF (200 ml) at 0°C. The mixture was stirred at room temperature for one hr and then cooled to 0°C. Water (8 ml), 15% aqueous NaOH solution (8 ml), and water (24 ml) were successively added. The cold mixture was filtered, the precipitate was washed with ethyl ether, and the filtrate was concentrated to afford a white solid of the crude diol (32.8 g, 89%).

Dimethyl sulfoxide (33.3 ml, 431 mm) dissolved in dry CH_2Cl_2 (100 ml) was added to a solution of oxalyl chloride (16.7 ml, 184 mm) in dry CH_2Cl_2 (450 ml) at -50° to -60°C . The mixture was stirred for 2 min, and the diol (32.7 g, 89.0 mm) in dry CH_2Cl_2 (170 ml) was added; stirring was continued for an additional 15 min. Triethylamine (125 ml) was next added, and the mixture was stirred for 5 min before being allowed to warm to room temperature. Water was then added and the aqueous layer was extracted with CH_2Cl_2 . The combined extracts were washed with saturated NaCl solution, dried over anhydrous MgSO_4 , and concentrated. The residue was chromatographed on silica gel with EtOAc–toluene (1:9) to afford an oil of **3** (32.2 g, 99%). NMR δ_{H} (90 MHz, CDCl_3): *c.* 1.5 (6H, m), 2.0 (4H, m), 2.92 (4H, d, $J=2$ Hz, OH), 3.34 (8H, s), 9.79 (2H, t, $J=2$ Hz). IR ν_{max} (film) cm^{-1} : 1735, 1715 (s). HR-MS m/z Found: M^+ 364.0677. Calcd. for $\text{C}_{15}\text{H}_{24}\text{O}_2\text{S}_4$: 364.0658.

Aldol condensation of 3 with iso-PrOAc or tert-BuOAc. To a THF (50 ml) solution of lithium diisopropylamide prepared from diisopropylamine (12.14 g, 120 mm) and *n*-butyllithium (hexane solution, 62.5 ml, 100 mm) was added dropwise a dry THF (50 ml) solution of iso-PrOAc (12.3 g, 120 mm) at -78°C over 45 min. After stirring for 10 min, a

solution of **3** (18.2 g 50 mm) in dry THF (40 ml) was added dropwise for 15 min. The mixture was stirred for 2 hr at -78°C , quenched with EtOH (20 ml), and warmed to ambient temperature. Water was added and the aqueous layer was extracted with diethyl ether. The ether extracts were washed with sat. NaCl solution and dried over MgSO_4 . The condensed filtrate was chromatographed on silica gel with EtOAc–*n*-hexane (3:7) to afford an oil of **4** (16.7 g, 62%). The *tert*-BuOAc-adduct **5** was also obtained quantitatively.

4. NMR δ_{H} (90 MHz, CDCl_3): 1.19 (6H, t, $J=7$ Hz), 2.50 (4H, d, $J=6$ Hz), 3.31 (8H, s), 3.69 (2H, d, $J=3$ Hz, OH), 4.17 (4H, q, $J=7$ Hz), 4.40 (2H, m). IR ν_{max} (film) cm^{-1} : 3450, 1725. HR-MS m/z Found: M^+ 540.1751. Calcd. for $\text{C}_{23}\text{H}_{40}\text{O}_6\text{S}_4$: 540.1706.

5. NMR δ_{H} (90 MHz, CDCl_3): 1.46 (9H, s), 2.42 (4H, d, $J=7$ Hz), 3.30 (8H, s), 3.70 (2H, d, $J=3$ Hz, OH), 4.36 (2H, m). IR ν_{max} (film) cm^{-1} : 3450, 1720. HR-MS m/z Found: M^+ 596.2318. Calcd. for $\text{C}_{27}\text{H}_{48}\text{O}_6\text{S}_4$: 596.2330.

3, 13-Di(tetrahydropyranyl)oxy-5,11-dioxopentadecadioic acid-5,11-di(ethylenedithio)ketal (8) To a solution of bis-ethyl ester **4** (16.7 g, 30.9 mm) and *p*-TsOH (445 mg) in dry dioxane (125 ml) was added dropwise dihydropyran (17.8 ml) with stirring at 10°C . After 20 min, powdered NaHCO_3 (*c.* 2 g) was added, and the filtrate was concentrated to afford a crude oil of **6**. Sodium hydroxide (12 g) was added to a solution of **6** in 80% aqueous MeOH (200 ml) at 0°C . After 2 hr of stirring at room temperature, the mixture was acidified with dilute HCl and extracted with EtOAc. The combined extract was washed with water, dried over MgSO_4 , and concentrated. The residue was chromatographed on silica gel, and elution with EtOAc–toluene (3:7) gave a slightly yellow powder of **8** (15.5 g, 77%). NMR δ_{H} (90 MHz, CDCl_3): *c.* 2.7 (4H, m), 3.27 (8H, s), 4.76 (2H, br s), *c.* 8.0 (2H, br s, COOH). IR ν_{max} (film) cm^{-1} : 3100, 1720 (s), 1705. FD-MS m/z : 652 (M^+).

1,15-Dihydroxy-3,13-di(tetrahydropyranyl)oxy-5,11-dioxopentadecane-5,11-di(ethylenedithio)ketal (9) A crude oil of **7** was obtained by the same method as that described for **6**. Reduction of **7** with LiAlH_4 was done by the same method as that described for **3**, giving an oil of **9** (quantitative yield). NMR δ_{H} (90 MHz, CDCl_3): 3.27 (8H, s), *c.* 4.7 (2H, m). IR ν_{max} (film) cm^{-1} : 3400. HR-MS m/z Found: M^+ 624.2640. Calcd. for $\text{C}_{29}\text{H}_{52}\text{O}_6\text{S}_4$: 624.2643.

Esterification of 8 with each alcohol (9, 10, or 1,3-propanedial) with subsequent removal of the THP ether protecting groups. To a solution of **8** (2.62 g, 4.02 mm), **10** (2.79 g, 8.04 mm) and 4-DMAP (196 mg, 1.6 mm) in dry CH_2Cl_2 (40 ml) was added *N,N*-dicyclohexylcarbodiimide (DCC, 1.82 g, 8.84 mm). The mixture was concentrated after 18 hr of stirring at room temperature, and the residue was chromatographed on silica gel with EtOAc–*n*-hexane (2:8) to afford an oil of **11** (2.37 g, 45%). A solution of **11**

(2.18 g, 1.66 mm) in acetic acid-THF-water (4:2:1, 70 ml) was stirred at 45°C for 6 hr and concentrated. The residue was chromatographed on silica gel, and elution with MeOH-CH₂Cl₂ (2:98) gave a gum of **12** (1.53 g, 94%).

Condensation of **8** (5.31 g, 8.14 mm) with **9** (5.08 g, 8.14 mm), as already described, gave a crude product **13** (2.46 g, 24%) after purification by chromatography on silica gel with EtOAc-toluene (2:8). A solution of partially purified **13** (2.46 g, 1.98 mm) and 0.1 N HCl (0.9 ml) in EtOH (80 ml) containing toluene (10 ml) was refluxed for 15 min. Powdered NaHCO₃ was added and the filtrate was concentrated. The residue was chromatographed on silica gel, and elution with EtOAc-toluene (2:8) gave a gum of **14** (480 mg, 27%). Compound **16** (450 mg, 11%) as an oil was obtained through a similar procedure to that described for **14**.

12. NMR δ_H (90 MHz, CDCl₃): 0.89 (6H, br t), 2.35 (4H, t, $J=7$ Hz), 2.51 (4H, d, $J=6$ Hz), 3.31, 3.33 (each 8H, s), 3.16, 3.70 (each 2H, d, $J=3$ Hz, OH), 3.96 (2H, m), c. 4.4 (6H, m). IR ν_{max} (film) cm⁻¹: 3450, 1725. FD-MS m/z : 976 (M⁺).

14. NMR δ_H (90 MHz, CDCl₃): 2.53 (4H, d, $J=6$ Hz), 3.32 (16H, s), 3.60, 3.70 (each 2H, br. s, OH), 4.29 (4H, t. like), c. 4.1, 4.4 (each 2H, m). IR ν_{max} (film) cm⁻¹: 3450, 1725. FD-MS m/z : 905 (M⁺ + H).

16. NMR δ_H (90 MHz, CDCl₃): c. 2.1 (8H, m), 2.52, 2.54 (each 2H, d, $J=7$ Hz), 3.31 (8H, s), 3.70 (2H, d, $J=3$ Hz, OH), 4.28 (4H, t, $J=6$ Hz), 4.38 (2H, m). IR ν_{max} (film) cm⁻¹: 3450, 1730. HR-MS m/z Found: M⁺ 524.1411. Calcd for C₂₂H₃₆O₆S₄: 524.1393.

PML-777 and cyclic analogues. A suspension of **12** (1.53 g, 1.57 mm), mercuric chloride (3.65 g) and mercuric oxide (1.13 g) in water (4.8 ml) and acetonitrile (80 ml) was stirred for 4 hr at 20°C. The cooled mixture was filtered and the precipitate was washed with chloroform. The filtrate was washed with water, saturated ammonium chloride solution and again with water. Evaporation of the solvent and subsequent precipitation from acetone in *n*-hexane gave a colorless powder of PML-777 (690 mg, 60%).

Two cyclic analogues were also similarly obtained.

PML-777. NMR δ_H (400 MHz, CDCl₃): 0.89 (6H, t, $J=7$ Hz), c. 1.38~1.53 (4H, m), 1.57 (4H, quint, $J=7$ Hz), 2.48 (2H, d. like, $J=7$ Hz), 2.48 (2H, d. like, $J=6$ Hz), 2.55 (2H, dd, $J=17, 8$ Hz), c. 2.59 (2H, dd, $J=17, 4$ Hz), c. 2.60 (2H, dd, $J=17, 3$ Hz), 2.65 (2H, dd, $J=17, 8$ Hz), 2.79 (4H, t, $J=6$ Hz), 2.86, 2.88 (each 1H, br. d, OH), 3.45, 3.49 (each 1H, d, $J=2$ Hz, OH), 4.07 (2H, br. s), 4.38 (4H, m), 4.44 (2H, m). IR ν_{max} (KBr) cm⁻¹: 3400, 1720 (s), 1715. FD-MS m/z : 695 (M⁺ + Na). Anal. Found: C, 61.97; H, 8.95. Calcd. for C₃₅H₆₀O₁₂·1/2H₂O: C, 61.67; H, 8.96%.

PMC₃₂-7777. A colorless powder (135 mg, 42%) from acetone in *n*-hexane. NMR δ_H (400 MHz, CDCl₃): 1.28 (8H, m), 1.60 (8H, m), 1.77 (4H, m), 2.46, 2.47 (each 4H, t, $J=7$ Hz), 2.53, 2.59 (each 4H, d. like, $J=7$ Hz), 2.63 (2H, dd, $J=17, 5$ Hz), 2.68 (2H, dd, $J=17, 7$ Hz), 3.40 3.66 (each 2H, m, OH), 4.18, 4.47 (each 2H, m). IR ν_{max}

(KBr) cm⁻¹: 3400, 1720 (s), 1715 (s), 1700. FD-MS m/z : 623 (M⁺ + Na), 601 (M⁺ + H). Anal. Found: C, 58.77; H, 7.88. Calcd. for C₃₀H₄₈O₁₂·1/2H₂O: C, 59.11; H, 8.05%.

PMC₂₀-711. A slightly yellowish solid (167 mg, 52%) after purification by chromatography on silica gel with EtOAc-toluene (3:7). NMR δ_H (400 MHz, CDCl₃): 1.25 (2H, m), 1.62 (4H, m), 2.00 (2H, quint, $J=5$ Hz), 2.45 (4H, t, $J=7$ Hz), 2.54 (2H, dd, $J=16, 6$ Hz), 2.58 (2H, dd, $J=16, 5$ Hz), 2.65 (2H, dd, $J=17, 5$ Hz), 2.73 (2H, dd, $J=17, 7$ Hz), 3.49 (1H, d, $J=2$ Hz, OH), 3.50 (1H, d, $J=3$ Hz, OH), 4.25 (4H, m), 4.46 (2H, m). IR ν_{max} (KBr) cm⁻¹: 3500, 3400, 1720, 1705. HR-MS m/z Found: M⁺ - H₂O, 354.1626. Calcd. for C₁₈H₂₆O₇: 354.1676.

Isolation of mitochondria and bioassays. The methods for the isolation of mitochondria and NADH oxidation assays have been reported previously.¹³⁾

Acknowledgments. The authors thank Dr. J. Uzawa, Mrs. T. Chijimatsu and Mr. Y. Esumi of the Institute of Physical and Chemical Research for NMR and mass spectra. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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