MASS SPECTROMETRY OF PLATENOLIDES AND THEIR DERIVATIVES IN CONNECTION WITH STRUCTURE ELUCIDATION

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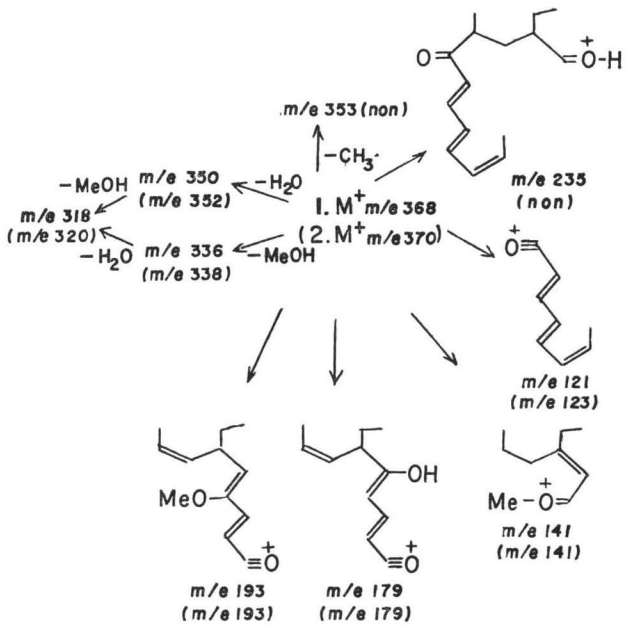
Mass spectra of platenolides—biosynthetic precursors of the 16-membered macrolide antibiotics, platenomycins—and their derivatives are discussed in detail especially in connection with structure elucidation. Mass spectrometry was of great use in establishing the structures for platenolides I (**1**) and II (**2**).

During the course of studies on the biosynthesis of the platenomycins, 16-membered macrolide antibiotics, platenolides I and II, biosynthetic precursors of the aglycone, were isolated from the fermentation beer of blocked mutant strains of *Streptomyces platensis* subsp. *malvinus* MCRL 0388. The structures of platenolides I and II have been proposed as **1** and **2** respectively.[1~4] In the present paper, the authors will discuss the mass spectra of the platenolides and their derivatives in detail, especially in connection with their structure elucidation. Structures of the platenolides and their derivatives dealt within this paper are illustrated in Fig. 1a and 1b.

The electron impact (EI) and the chemical ionization (CI) mass spectra of **1** and **2** are illustrated in Fig. 2a and 2b.

In EI mass spectra, molecular ions were observed at *m/e* 368.2196 ($C_{20}H_{32}O_6$, Δ 0.1 mmu) for **1** and *m/e* 370.2301 ($C_{20}H_{34}O_6$, Δ −5.1 mmu) for **2**. Adjacent fragment ions resulting from the elimination of water and methanol support the presence of hydroxyls and a methoxyl. Partial structures common to **1** and **2** were suggested by the ions at *m/e* 193, 179 and 141, which result from the macrocyclic lactone ring according to the cleavage shown in Scheme 1.

Scheme 1.
Parentheses are for **2**.



Fragment peaks at *m/e* 235 and 121 of **1** were also related to the lactone ring and involved the C-9 carbonyl function. The compositions

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of their ions were determined by high resolution as shown in Table 1.

In CI spectra of **1** and **2**, intense quasimolecular ions appeared respectively at *m/e* 369 (QM^+, M+H) and 369 (QM^+, M−H) together with subsequent dehydration peaks respectively at *m/e* 351 (369−H_2O) and 353 (370−OH) resulting from the different chromophores. However, no fragment ions suggesting the relation of any oxygen functions were observed with either method of mass spectroscopy.

On acetylation with acetic anhydride and pyridine at room temperature, **1** afforded a diacetate (**3**)[1] which showed a molecular ion at *m/e* 452.2390 ($C_{24}H_{36}O_8$, Δ −1.8 mmu). Several signals in the ^{1}H-NMR spectrum of **3** were assigned to specific hydrogens by means of proton decoupling experiments (Fig. 3.).

Signals of H-3 and H-5 of **1** shifted to the lower field in **3**.

Investigation of the 3,5-diol system by the decoupling method showed that the coupling constants of H-3, 4 and H-5, 6 were nearly zero and $J_{4,5}$ was 10.2 Hz. Thus the location of two hydroxyl and a methoxyl functions could not be determined by ^{1}H-NMR. Although **1** and **2** did not give any preferable acetonide derivative, the reaction of **1** with phenyl boronic acid under azeotropic conditions in acetone afforded an unstable phenyl boronate (**13**) as a slightly crude product; **13** gave an intensive molecular ion peak (*m/e* 454 in EI and *m/e* 455 (QM^+, M+H) in CI) and a characteristic boron-containing fragment ion at *m/e* 187 in EI as shown in Fig. 4 and in Scheme 2. These peaks were enough to ascertain 3,5-diol system of **1**.

Fig. 1a. The structures of platenolides and their derivatives and the reaction scheme.

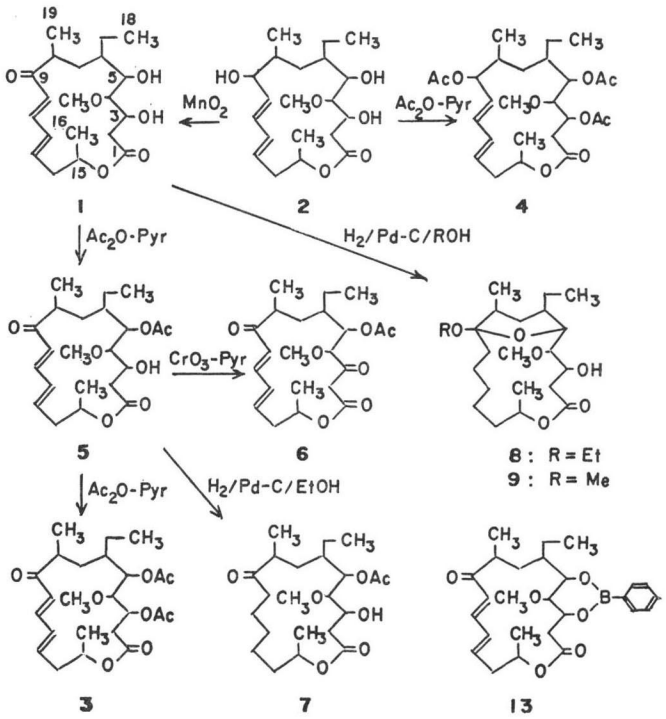


Fig. 1b. The structures of platenolide II (**2**) derivatives and the reaction scheme.

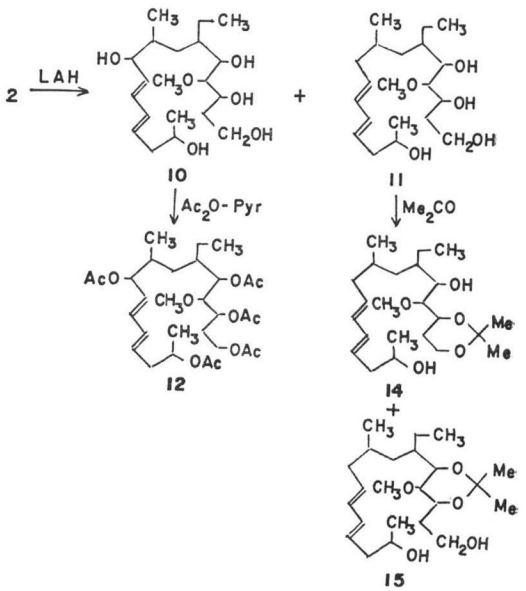


Fig. 2a. Mass spectra of platenolide I(**1**).

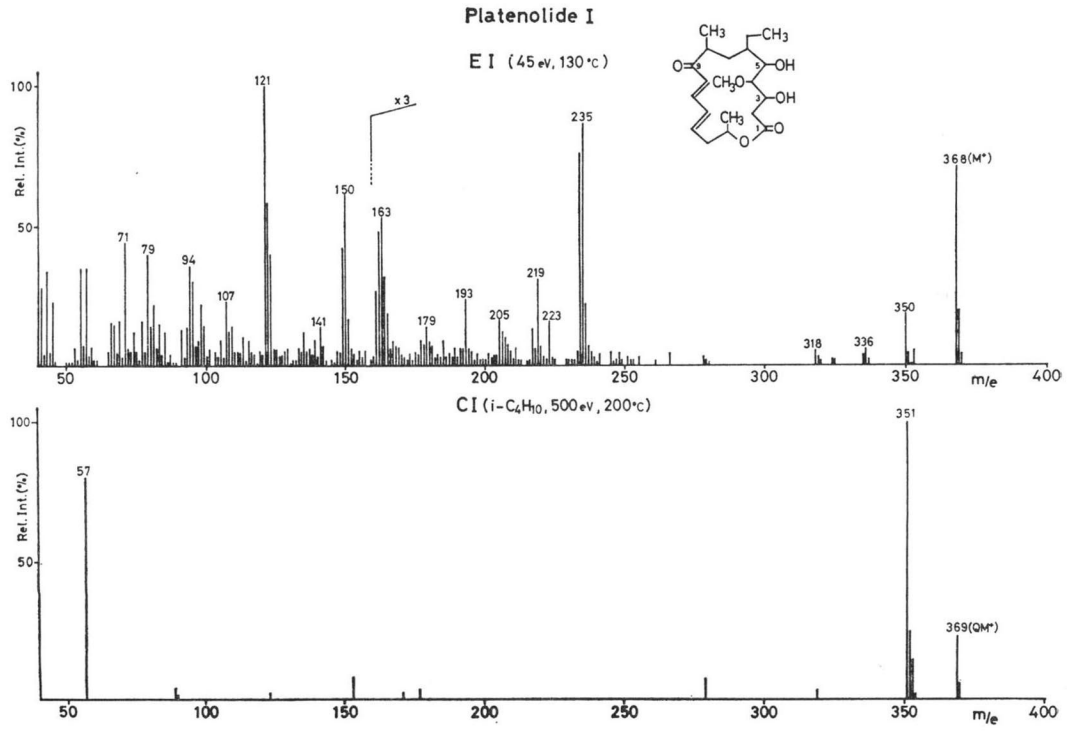


Fig. 2b. Mass spectra of platenolide II(**2**).

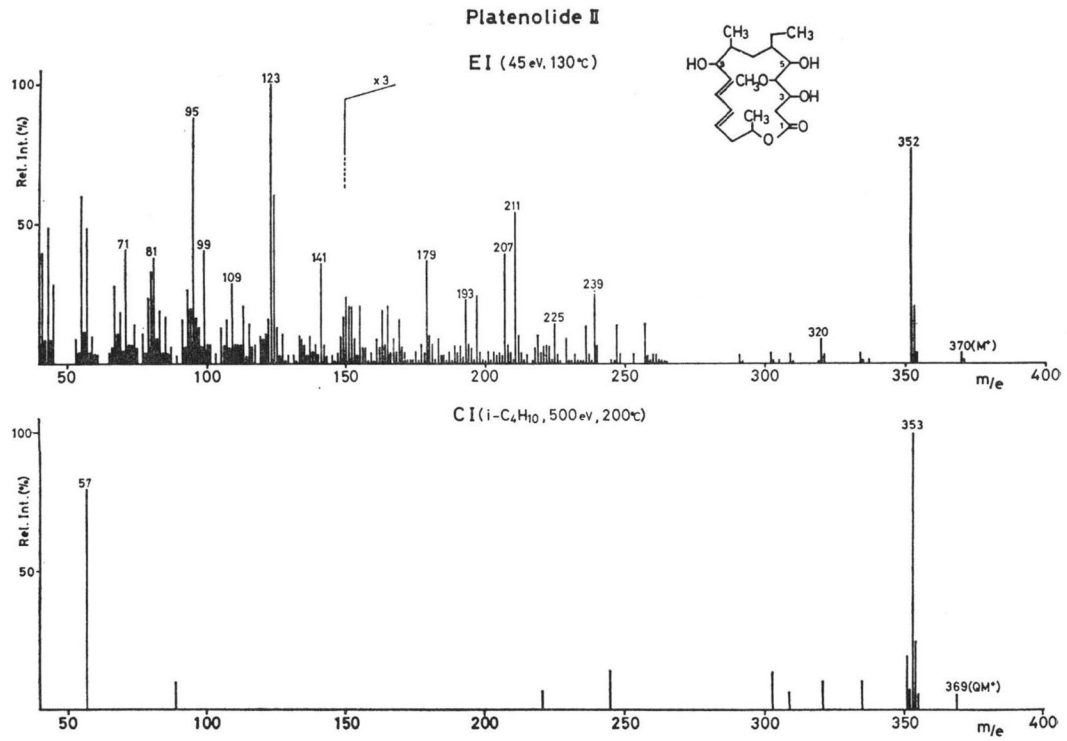
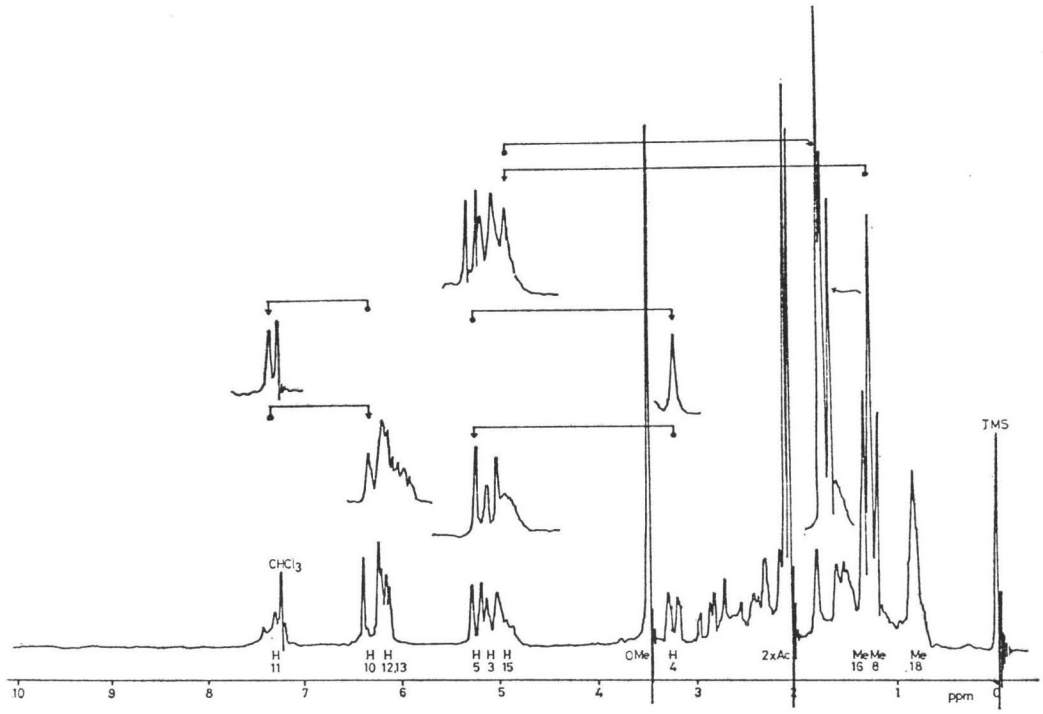


Table 1. Compositions of fragment ions of **1** and **2** determined by high resolution.

Platenolide I(**1**)			Platenolide II(**2**)		
obs. (*m/e*)	composition	error (mmu)	obs. (*m/e*)	composition	error (mmu)
368.2196	$C_{20}H_{32}O_6$	0.1	370.2301	$C_{20}H_{34}O_6$	−5.1
353.1936	$C_{19}H_{29}O_6$	−2.8	352.2274	$C_{20}H_{32}O_5$	2.4
350.2096	$C_{20}H_{30}O_5$	0.5	338.2122	$C_{19}H_{30}O_5$	2.9
336.1929	$C_{19}H_{28}O_5$	−0.6	320.1941	$C_{19}H_{28}O_4$	−4.7
318.1176	$C_{19}H_{26}O_4$	−5.6	193.1242	$C_{12}H_{17}O_2$	1.4
235.1694	$C_{15}H_{23}O_2$	−0.5	179.1037	$C_{11}H_{15}O_2$	−3.5
193.1217	$C_{12}H_{17}O_2$	−1.1	141.1299	$C_9H_{17}O$	2.2
179.1037	$C_{11}H_{15}O_2$	−3.5	123.0826	$C_8H_{11}O$	1.6
141.1299	$C_9H_{17}O$	2.2			
121.0688	C_8H_9O	3.5			

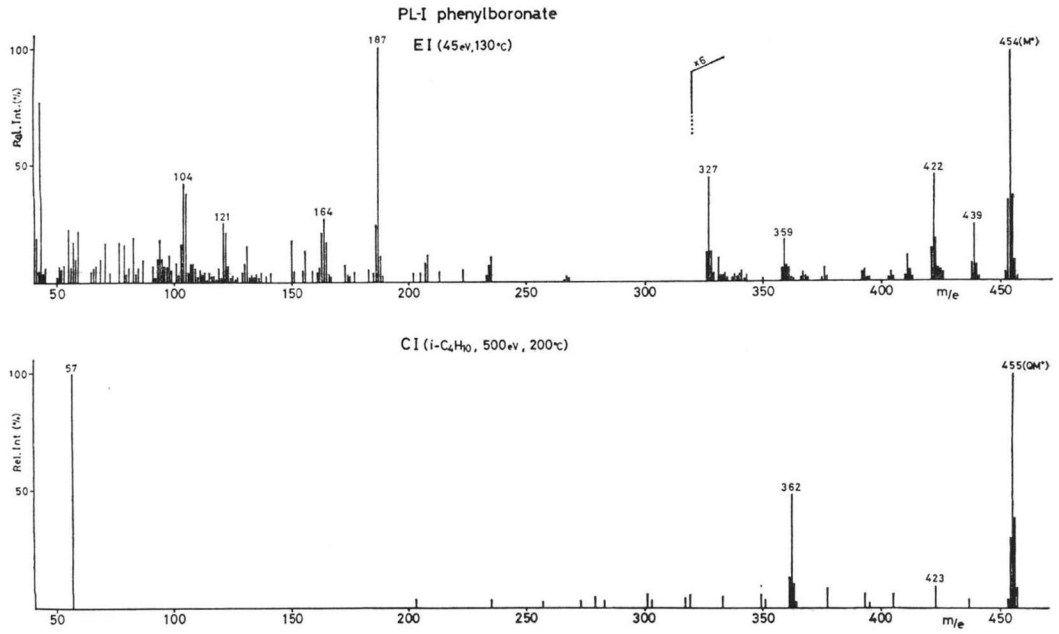
Fig. 3. ^{1}H-NMR spectrum and decoupling of diacetyl platenolide I(**3**).



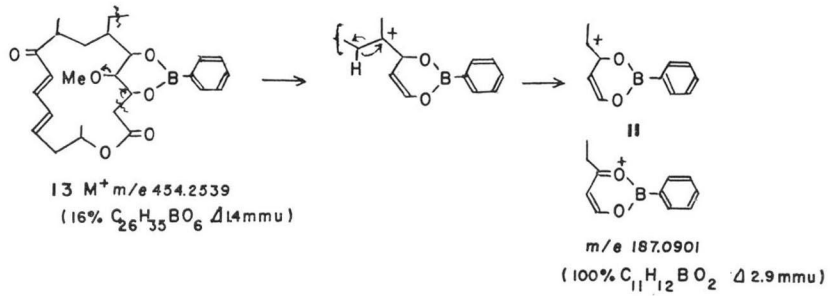
With acetic anhydride-pyridine **1** was acetylated at low temperature to a monoacetyl derivative (**5**, $C_{22}H_{34}O_7$) and **5** was oxidized with CrO_3 in pyridine to a dehydro compound (**6**, $C_{22}H_{32}O_7$) which showed similar UV absorption as **1** in neutral medium. In alkaline medium, however, **6** showed a strong UV maximum at 269 nm (log ϵ 4.80) which was ascribed to the enolate anion (–OCO–CH=$\overset{|}{C}$–O$^{\ominus}$) and reflected the 3-ketolactone structure.[5]

Catalytic reduction of **5** on Pd-C in ethanol gave a tetrahydro derivative (**7**, $C_{22}H_{38}O_7$) with end UV absorption. However, when **1** was directly reduced catalytically on Pd-C in ethanol or in methanol, any normal tetrahydrogenated product was not obtained, but compounds assumed to a tetrahydro ethyl ketal (**8**) or a tetrahydro methyl ketal (**9**) were obtained. Mass spectra of these reduction products

Fig. 4. Mass spectra of platenolide I phenyl boronate (**13**).



Scheme 2.



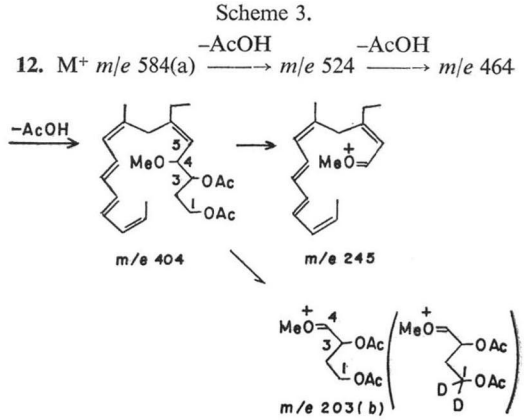
gave little information as to the structure of the original compound.

Total acetylation of **2** gave a triacetate (**4**)[1], which showed a molecular ion at *m/e* 496.2669 ($C_{26}H_{40}O_9$, Δ −1.9 mmu).

That **2** is a dihydro derivative of **1** was confirmed by the oxidation of **2** to **1**.[1] EI mass spectra of **3** and **4** showed several characteristic fragment peaks due to elimination of MeOH and CH_3COOH from M^+. In the low mass region the cracking peaks were nearly the same as the parent compounds and were of little use in determining partial structures.

Reduction of **2** with lithium aluminium

Scheme 3.

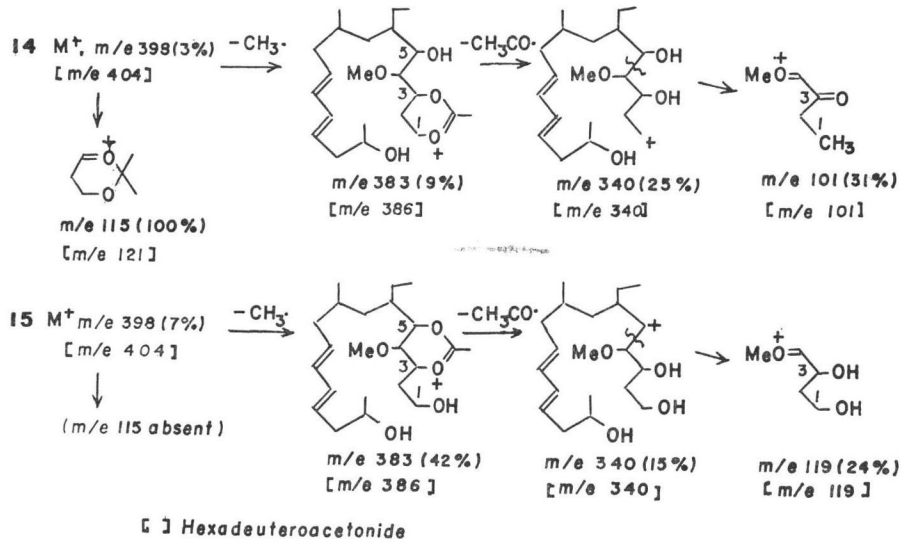


hydride in tetrahydrofuran resulted in two products, **10** and **11**. One (**10**) of them was a penta-alcohol possessing the original UV absorption resulting from the cleavage of the lactone ring, as determined by mass spectrometry of its penta-acetyl derivative (**12**). Fragment peaks due to stepwise elimination of three acetic acids from M^+ were observed in the higher mass region in the spectrum of **12**. A diagnostic peak (*m/e* 203) strongly characteristic of the C-1~C-4 position was also present. The structure of this ion was determined by its shift to *m/e* 205 in a lithium aluminium deuteride reduction product and to *m/e* 209 and 211 in trideuteroacetyl derivative (Scheme 3. and Table 2.).

Table 2. Fragment ions, a and b, in Scheme 3, shifted by lithium aluminium deuteride reduction and deuteroacetate.

1-position	acetate	ions (*m/e*)	
		a	b
H_2	CH_3CO	584	203
D_2	CH_3CO	586	205
H_2	CD_3CO	599	209
D_2	CD_3CO	601	211

Scheme 4.



Another reduction product of **2** was a tetra-alcohol (**11**) in which not only the lactone ring was cleaved, but the hydroxy function was hydrogenated. Based on the UV maximum at 220 nm, **11** was concluded to be a diene chromophore due to the lacking of 9-hydroxy function. The tetra-alcohol (**11**) gave two acetonide isomers (**14** and **15**) on treatment with acetone and anhydrous cupric sulfate. These acetonides exhibited the same molecular ion at *m/e* 398. The structure of **14** was characterized by the presence of an intense ion at *m/e* 115. The structures of **14** and **15** were confirmed by the presence of the fragment ions shown in Scheme 4. The mass spectra of these lithium aluminium hydride reduction products confirmed the position of oxygen functions in the structure of **2**.

Consequently, mass spectral analyses of the platenolides and their derivatives played an important role in elucidating the structures of the platenolides.

Experimental

General methods

Melting points were not corrected. The following instruments were used for measurements: Hitachi EPI-32 IR spectrometer, Hitachi-323 UV spectrometer, JEOL PS-100 spectrometer for 100 MHz ^{1}H-NMR in $CDCl_3$ solution with TMS as the internal standard, Hitachi RMS-4 spectrometer for low resolution MS, Shimadzu LKB-9000 spectrometer for chemical ionization MS, Hitachi RMU-7M or CEC 21-110B spectrometer for high resolution mass spectrometry. Errors (Δ) of high resolution mass spectra are presented by millimass units (mmu). Thin-layer chromatography was performed on silica gel GF_{254} (Merck) plate with thickness 0.25 mm and spots were visualized by spraying with 40% H_2SO_4 followed by heating.

Platenolides I (**1**) and II (**2**)

The platenolides (**1** and **2**) were extracted from the fermentation beer of blocked mutant strains of *Streptomyces platensis* subsp. *malvinus* MCRL 0388 and purified by column chromatography and recrystallization, as previously reported.[1]

5-O-Acetyl platenolide I (**5**)

Two hundred mg of **1** was dissolved in 5 ml acetone and 0.5 ml pyridine and 2 ml acetic anhydride were added. The reaction solution was allowed to stand at 5°C for 10 hours. After concentrating the reaction solution, the residue was chromatographed on a silica gel (Merck) column using the solvent system of benzene - acetone (95:5). The column effluent was monitered by TLC [silica gel, benzene - acetone (4:1), Rf: 0.45 for **5** and 0.75 for **3**]. The product **5** was collected and recrystallized from benzene-hexane to give 55 mg. m.p. 204~206°C. Anal. calcd. for $C_{22}H_{34}O_7$, C, 64.37; H, 8.34; obs. C, 64.35; H, 8.26. IR (nujol, cm^{-1}); 3530, 1735, 1710, 1690, 1635 and 1595. UV (EtOH); λ_{max} 280 nm (log ε 4.39). MS; *m/e* 410 (M^+), 395 (M—CH_3), 378 (M—MeOH), 350 (M—AcOH). ^{1}H-NMR; δ 2.08 (s, 3H, –$COCH_3$), 3.05 (bd, 1H, J=9.5, H–4), 3.72 (bd, 1H, J=10, H–3) and 5.49 (d, 1H, J=9.5, H–5).

Diacetyl platenolide I (**3**)

Diacetyl platenolide I (**3**) was produced by the previous method[1] or by further acetylation of **5** at room temperature. m.p. 180~181°C. Anal. calcd. for $C_{24}H_{36}O_8$; C, 63.70; H, 8.02. obs. C, 63.78; H, 8.01. IR (nujol); 1755, 1685, 1635, 1600, 1315, 1240 and 1190. UV (EtOH); λ_{max} 279.5 nm (log ε 4.40). MS; *m/e* 452 (M^+), 437 (M—CH_3), 392 (M—AcOH) and 333 (M—AcOH—AcO). ^{1}H-NMR; δ 2.05 (s, 3H, CH_3CO–), 2.08 (s, 3H, CH_3CO–).

Oxidation of 5-O-acetyl platenolide I (**5**)

Five mg of **5** was stirred in 0.5 ml acetone with 5 mg CrO_3 in an ice bath, one drop of pyridine was added, and the mixture stirred for 12 hours. The product (**6**) appeared at Rf 0.60 compared with 0.45 for **5** on TLC (silica gel, benzene - acetone (3:1)). The product (**6**) was isolated by preparative TLC. m.p. 173~175°C. UV (EtOH); λ_{max} 275 nm (log ε 4.38), (alkali-EtOH); 269 nm (log ε 4.80). MS; *m/e* 408.2171 (M^+, $C_{22}H_{32}O_7$, Δ 3.2 mmu), 393 (M—CH_3), 376 (M—MeOH) and 348 (M—AcOH).

Reduction of 5-O-acetyl platenolide I (**5**)

In 3 ml ethanol, 40 mg of **5** was catalytically reduced using 10 mg of 5% Pd-C catalyst at room temperature and atmospheric pressure. After 2 hours, the solution was filtered and concentrated to dryness. The residue was recrystallized from ether - hexane to afford 20 mg of colorless needles of **7**. m.p. 135~136°C. Anal. calcd. for $C_{22}H_{38}O_7$; C, 63.74; H, 9.24; obs. C, 63.78; H, 9.08. IR (nujol, cm^{-1}); 3420, 1740, 1735 and 1710. UV (EtOH); end absorption. MS; *m/e* 414 (M^+), 399 (M—CH_3), 382 (M—MeOH) and 354 (M—AcOH).

Catalytic reduction of platenolide I (**1**)

Under atmospheric pressure at room temperature, 100 mg of **1** was catalytically reduced in 5 ml ethanol using 20 mg of 5% Pd-C for 2 hours. The reaction solution was filtered and concentrated to dryness to yield 100 mg of oily product (**8**) which contained no starting material. MS; *m/e* 400 (M^+), 355 (M—$C_2H_5O\cdot$). IR (nujol, cm^{-1}); 3420, 1740. UV (EtOH); end. ^{1}H-NMR; δ 0.87 (t, 3H, J=6, CH_3—18), 0.90 (t, 3H, J=6, $C\underline{H}_3CH_2O$–), 3.61 (q, 2H, J=6, $CH_3C\underline{H}_2O$–) and ~1.6 (m, 4H). It was hard to assign the protons at 3, 4, 5 and 9 positions.

Lithium aluminium hydride reduction of platenolide II (**2**)

To the suspension of 1.1 g of lithium aluminium hydride in 20 ml of tetrahydrofuran, 500 mg of **2** dissolved in 4 ml of tetrahydrofuran were added dropwise, then refluxed for 20 hours. Water was added and the reaction mixture extracted with EtOAc. The extract was dried over anhydrous Na_2SO_4 and concentrated to oil. The residue was chromatographed on a silica gel (45 g, Merck) column using the solvent system of benzene - acetone (3:2). The tetra-alcohol (**11**) eluted first and was recrystallized from isopropylether to give 123 mg of crystals. The penta-alcohol (**10**, 70 mg) was subsequently eluted as an oil.

11, tetra-alcohol; m.p. 54~55°C. Anal. calcd. for $C_{20}H_{38}O_5$; C, 67.00; H, 10.68, obs. C, 66.36; H: 10.61. IR (nujol. cm^{-1}); 3250. UV(EtOH;); λ_{max} 220 nm (log ε 4.28). ^{1}H-NMR; δ 0.86 (t, 3H, J=6, CH_3-18), 1.09 (d, 3H, J=6, -CH_3), 1.16 (d, 3H, J=6, -CH_3). 3.55 (s, 3H, -OCH_3), 5.1~6.4 (m, 4H, vinylic). MS; *m/e* 358 (M^+, 1.2%), 340($M-H_2O$, 5%), 308(340−MeOH, 2.4%).

10, penta-alcohol; oil at room temperature. IR(neat, cm^{-1}); 3250. This compound was acetylated by pyridine - acetic anhydride to penta-acetate (**12**); oil at room temperature. IR($CHCl_3$, cm^{-1}); 1730, 1240. UV (EtOH); λ_{max} 234 nm(log ε 4.35). MS; *m/e* 584.3206(M^+, 8%, ion *a*, $C_{30}H_{48}O_{11}$, Δ 1.2 mmu), 552 (*M*−MeOH, 26%), 404.2579(M−3×AcOH, 31%, $C_{24}H_{36}O_5$, Δ 1.5 mmu), 245.1814 (18%, $C_{17}H_{25}O$, Δ−1.9 mmu) and 203.0919 (78%, ion *b*, $C_9H_{15}O_5$, Δ 0.1 mmu). ^{1}H-NMR; δ 0.88 (t, 3H, J=6, CH_3-18), 0.91 (d, 3H, J=6, CH_3-19), 1.20 (d, 3H, J=6, CH_3-16), five acetyl methyls at 2.02, 2.04, 2.05, 2.10 and 2.12, and 3.50 (s, 3H, -OCH_3).

Lithium aluminium deuteride reduction was carried out by the same procedure using $LiAlD_4$ and the trideuteroacetate was prepared with $(CD_3CO)_2O$ and pyridine.

Acetonides (**14** and **15**) of tetra-alcohol (**11**)

For 2 hours, 111 mg of **11** in 5 ml of anhydrous acetone was refluxed with 100 mg of anhydrous cupric sulfate. Cupric sulfate was filtered off and the filtrate was concentrated to yield a yellowish oil (140 mg). The oil was chromatographed on a silica gel (10 g, Merck) column using benzene - acetone (3:2), which separated **14** (5 mg) and **15** (55 mg).

14; oil at r.t. IR($CHCl_3$, cm^{-1}) 3620, 3500. UV(EtOH); 224 nm (log ε 4.13). ^{1}H-NMR; δ 1.47(s, 6H, $(CH_3)_2C<^{O-}_{O-}$). MS; *m/e* 398.3047 (M^+, 8% $C_{23}H_{42}O_5$, Δ1.7 mmu), 383.2805 (M−CH_3, 26%, $C_{22}H_{39}O_5$, Δ 1.0 mmu), 340.2630 (383−$CH_3CO\cdot$, 20%, $C_{20}H_{36}O_4$, Δ1.8 mmu), 115.0748 (100%, $C_6H_{11}O_2$, Δ −0.9 mmu) and 101.0590 (31%, $C_5H_9O_2$, Δ−1.1 mmu).

15; oil at room temperature. IR ($CHCl_3$, cm^{-1}); 3620, 3500. UV (EtOH); 225 nm (log ε 4.13). ^{1}H-NMR; δ 1.47 (s, 6H, $(CH_3)_2C<^{O-}_{O-}$). MS; *m/e* 398.3006 (M^+, 8%, $C_{23}H_{42}O_5$, Δ−2.3 mmu), 383.2812 (M−CH_3, 43%, $C_{22}H_{39}O_5$, Δ 1.7 mmu), 340.2605 (383−$CH_3CO\cdot$, 15%, $C_{20}H_{36}O_4$, Δ−0.6 mmu) and 119.0754 (24%, $C_5H_{11}O_3$, Δ 4.9 mmu).

Hexadeuteroacetonides were made as above using $(CD_3)_2CO$.

Phenyl boronate (**13**) of platenolide I (**1**)

A solution of 123 mg of **1** and 60 mg of phenyl boronic acid in 30 ml of acetone was refluxed azeotropically for 5 hours. The reaction mixture was evaporated to dryness, and the residue passed through a sephadex LH-20 (Pharmacia) column using $CHCl_3$. The major peak was separated by preparative TLC, which yielded **13** as an amorphous powder (28 mg). m.p. 58~63°C. IR (nujol, cm^{-1}); 3500, 1730, 1680, 1640, 1600, 1440, and 1310. UV (EtOH); 280 nm (log ε 4.41). MS; *m/e* 454.2539 (M^+, 15%, $C_{26}H_{35}BO_6$, Δ 1.4 mmu), 439 (M−CH_3, 3%), 442 (M−MeOH, 5%) and 187.0901 (100%, $C_{11}H_{12}BO_2$, Δ−2.9 mmu).

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