STRUCTURES OF NEW 18-MEMBERED MACROLIDES FD-891 AND FD-892

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Structures of FD-891 and FD-892 were determined by extensive NMR spectral analysis as shown in Fig. 1. They belong to such 18-membered macrolides as concanamycins and virustomycin.

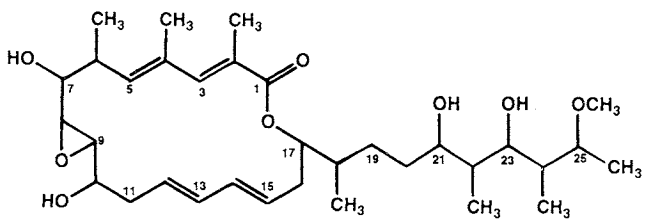
In the course of our screening program for low molecular substances inducing morphological changes of HL-60, new 18-membered macrolides FD-891 and FD-892 were discovered in the fermentation broth of *Streptomyce graminofaciens* A-8890[1]. They belong to such 18-membered antibiotics as concanamycins[2,3] and virustomycin[4,5]. Their biological properties are similar to those of 18-membered antibiotics[1]. Their structures were determined by extensive NMR analysis as shown in Fig. 1. This paper deals with their structural elucidation.

Results and Discussion

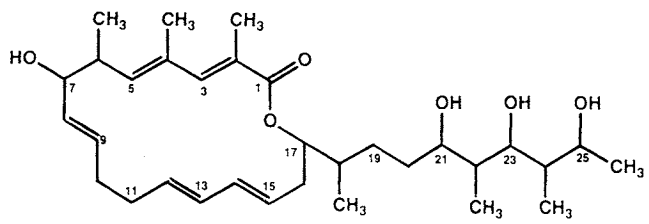
Structure of FD-891

The physico-chemical properties of FD-891 are shown in Table 1. The UV spectrum of FD-891 showed the absorption at 270 nm due to a diene system conjugated with a carbonyl group. The bands at 3400 and

Fig. 1. Structures of FD-891 and FD-892.



FD-891



FD-892

Table 1. Physico-chemical properties of FD-891 and FD-892.

	FD-891	FD-892
Appearance	White powder	White powder
MP (°C)	68.5~72	55~62
$[\alpha]_D$	+14.0° (*c* 0.1, MeOH)	+48.0° (*c* 0.05, MeOH)
FAB-MS (*m/z*)	579 $(M+H)^+$	533 $(M+H)^+$
HREI-MS (*m/z*)		
Found:	578.3827	532.3759
Falcd:	578.3819 for $C_{33}H_{54}O_8$	532.3764 for $C_{32}H_{52}O_6$
UV λ_{max}^{MeOH} (ε) nm	208 (5,060), 270 (12,480)	207 (7,710), 275 (14,040)
IR ν_{max}^{KBr} cm^{-1}	3400, 1710	3400, 1705

1710 cm^{-1} in the IR spectrum were assigned to a hydroxyl and an ester carbonyl, respectively; the existence of the latter was confirmed by the signal at δ_C 168.9 in the ^{13}C NMR spectrum.

The molecular formula was determined to be $C_{33}H_{54}O_8$ by the HREI-MS measurment of its molecular ion at 578.3827 (calcd. 578.3819) in connection with its NMR spectra. The degree of unsaturation was estimated to be 7 by its molecular formula. Four unsaturations were assigned to four double bonds and one to a carbonyl group, leaving the final two unsaturation to accommodate two rings. Only 50 hydrogens could be identified through the DEPT spectra described below, indicative of the existence of four secondary hydroxyl groups by its molecular formula. The four acetyl methyl signals at δ_H 2.10, 2.11, 2.31 and 2.33 in the ^1H NMR spectrum of the tetra acetyl derivative of FD-891 confirmed the existence of four secondary hydroxyl groups in the molecule.

The ^{13}C NMR spectrum of FD-891 showed 33 signals, consistent with its molecular formula, which were classified into $CH_3 \times 7$, $-CH_2- \times 4$, $>CH- \times 4$, $-OCH_3 \times 1$, $-CHO- \times 8$, $-CH= \times 6$, $>C= \times 2$ and $>C=O \times 1$ by its DEPT spectrum.

The ^1H and ^{13}C NMR data are summarized in Table 2.

The initial assignment of the partial structures was based mainly on the mapping information found in the ^1H-^1H COSY spectrum. Though signals due to unequivalent methylenes were overlapped in the high field region, they could be unambiguously assigned on the one-to-one basis by the aid of the ^1H-^{13}C COSY spectrum.

The fragment (1) in Fig. 2 was deduced by tracing the correlation maps from the cross peak at δ_H 4.17 (H-7) through δ_H 3.12 (H-6) to those at δ_H 5.55 (H-5), 1.15 (6-Me) and through δ_H 3.25 (H-8) to 3.15 (H-9) in the ^1H-^1H COSY spectrum. The signals for H-8 and H-9 showed correlation peaks with the lines at δ_C 55.1 and 56.0, respectively, in the ^1H-^{13}C COSY spectrum; their $^1J_{C-H}$ coupling values were estimated to be *ca.* 170 Hz. The trends of their ^{13}C chemical shifts and the measurement of their $^1J_{C-H}$ coupling constant values suggested C-8 and C-9 to be an epoxide ring as shown in Fig. 2.

The signals at δ_H 3.88 (H-10) made up a cross peak with the equivalent methylene at δ_H 2.55 (H_2-11), which in turn coupled to the olefinic proton at δ_H 5.64 (H-12). Thus, the fragment (2) was assigned as depicted in Fig. 2.

The signal at δ_H 4.87 (H-17) coupled to the unequivalent methylene protons at δ_H 2.20 and 2.45 (H-16 A and B), which showed cross peaks with an olefinic proton line at δ_H 5.75 (H-15) forming a correlation peak with the line at δ_H 5.60 (H-14). These results revealed the fragment (3) as shown in Fig. 2.

The analysis of the ^1H-^1H COSY spectrum in the same way led to the assignment of the fragments

Table 2. ^{1}H and ^{13}C chemical shifts of FD-891 and FD-892 measured in $CDCl_3$.

No.	FD-891		FD-892	
	δ_C	δ_H	δ_C	δ_H
1	168.9		169.8	
2	124.3		123.0	
3	144.0	7.30 (t; 1.3)	144.4	7.15 (t; 1.2)
4	135.7		132.3	
5	141.6	5.53 (br d; 10.3)	144.4	5.37 (t; 1.3)
6	35.9	3.12 (m; 10.3, 6.9, 4.1)	38.8	2.55 (m; 10.3, 7.0, 3.5)
7	70.8	4.17 (dd; 4.1, 6.2)	78.9	3.85 (m)
8	55.1	3.25 (dd; 6.0, 2.5)	125.5	5.34 (dd; 9.5, 15.8)
9	56.0	3.15 (dd; 2.5, 0.8)	133.2	5.78 (ddd; 16.0, 7.0, 9.2)
10	71.1	3.55 (m)	29.9	1.95 (m), 2.01 (m)
11	37.9	2.55 (m)	35.6	2.21 (m)
12	128.2	5.64 (m)	128.9	5.41 (m)
13	129.3	5.55 (m)	132.8	5.46 (m)
14	130.1	5.60 (m)	133.2	5.33 (m)
15	129.0	5.75 (ddd; 4.5, 10.2, 15.2)	131.9	5.55 (ddd; 10.0, 15.2, 6.0)
16	34.4	2.20 (ddd; 14.5, 4.5, 7.5), 2.45 (ddd; 14.5, 10.2, 4.5)	34.2	1.80 (ddd; 10.2, 5.0, 14.1), 2.20 (m)
17	76.7	4.87 (ddd; 7.5, 4.5, 2.5)	75.6	4.85 (ddd; 10.0, 2.5, 5.0)
18	34.4	1.85 (m)	37.0	1.78 (m)
19	35.9	1.95 (m), 2.25 (m)	36.4	1.92 (m), 2.20 (m)
20	36.7	2.15 (m), 2.30 (m)	36.3	1.90 (m), 2.17 (m)
21	73.3	3.88 (ddd; 10.0, 7.8, 9.0)	74.2	3.85 (m)
22	39.5	1.80 (m; 7.0, 8.1, 1.4)	39.0	1.96 (m)
23	78.2	3.82 (dd; 8.1, 1.4)	78.9	3.85 (m)
24	39.5	1.60 (m; 7.0, 9.5, 8.2)	39.5	1.50 (m; 6.5, 7.0, 10.5)
25	82.5	3.60 (m; 6.8, 9.5)	72.8	4.10 (m; 7.0, 10.5, 1.5)
$-OCH_3$	56.0	3.34		
2-CH_3	13.6	2.10 (d; 1.2)	13.6	2.03 (d; 1.3)
4-CH_3	15.5	2.03 (d; 1.2)	15.4	1.90 (d; 1.2)
6-CH_3	16.5	1.15 (d; 6.9)	17.2	1.16 (d; 6.9)
18-CH_3	16.3	0.88 (d; 7.0)	15.0	0.95 (d; 7.0)
22-CH_3	11.4	0.80 (d; 7.1)	11.9	0.79 (d; 7.1)
24-CH_3	5.0	0.90 (d; 7.0)	3.9	0.92 (d; 7.1)
25-CH_3	16.2	1.20 (d; 6.8)	21.3	1.19 (d; 7.0)

(4), (5), (6) and (7), respectively as shown in Fig. 2. The ambiguity due to the signal overlapping did not allow us to correctly assign and expand the partial structures in the ^{1}H-^{1}H COSY spectrum. To overcome these problems, the HMBC[6] technique was applied. As the peak heights of proton signals are known to be proportional to the intensities of the cross peaks of long range couplings between ^{1}H and ^{13}C observed in the heteronuclear multiple-bond correlation (HMBC) spectrum, the HMBC experiment is a particularly effective method for structural determination of the molecule with many methyl groups[7]. Therefore, the assignments of the partial structures substituted with methyl groups, the elucidation of the structures replaced with singlet methyls and the connectivities of these partial structures can be performed by the use of the HMBC technique.

The singlet methyl (2-Me) at δ_H 2.10 showed the cross peaks with C-1 (δ_C 168.9), C-2 (δ_C 124.5) and C-3 (δ_C 144.0), respectively. Furthermore, the signal for C-3 coupled with the singlet methyl (4-Me) at δ_H 2.03, which in turn correlated to C-4 (δ_C 135.7) and C-5 (δ_C 141.6). A ^{13}C-^{1}H long range coupling was observed between the carbon line due to C-5 and the doublet methyl (6-Me) at δ_H 1.15, which showed

Fig. 2. Structural fragments elucidated by tracing the cross peaks in the ^1H-^1H COSY spectrum of FD-891.

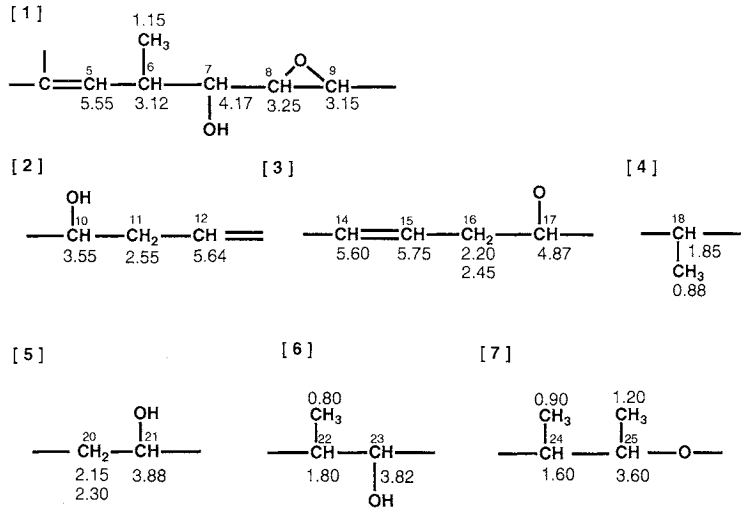
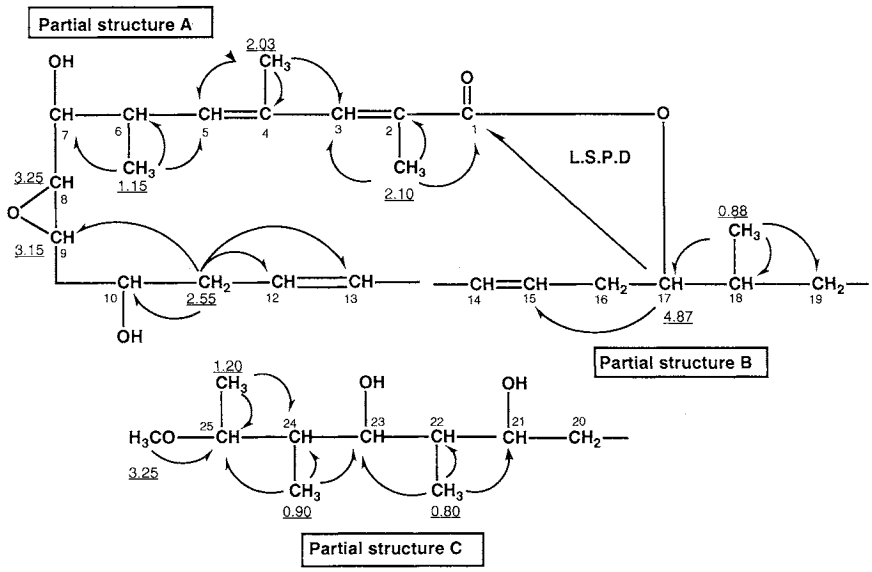


Fig. 3. Proton-carbon long range couplings detected by the HMBC method.



correlations with C-6 (δ_C 35.9) and C-7 (δ_C 70.7). Analysis of the HMBC spectrum allowed the partial structure from C-1 to C-5 to be assigned and connected with the fragment (1).

Long range cross peaks from almost equivalent methylen protons for H-11A and B at δ_H 2.55, which showed a A2 type coupling pattern, to C-13 (δ_C 129.3) and C-9 (δ_C 56.0) made it possible to combine between the fragment (1) and the fragment (2), revealing the partial structure A as shown in Fig. 3.

The fragment (4) was expanded and connected with the fragment (3) by tracing ^1H-^{13}C long range couplings from the doublet methyl (18-Me) at δ_H 0.88 to the carbon signals for C-17 (δ_C 76.7), C-18 (δ_C 34.4) and C-19 (δ_C 35.9), by which the partial structure B was deduced as depicted in Fig. 3. The signal

at δ_H 4.87 corresponding to H-17 did not shift downfield in the ^1H NMR of the acetyl derivative of FD-891, although the downfield shifts by *ca.* 1.2~1.8 ppm of the lines due to H-7, H-10, H-21 and H-23 were observed. An ester bond was determined to be formed by linking C-17 to C-1 as shown in Fig. 3, which could be confirmed by the observation of collapse of the signal for C-1 from a multiplet to a quartet at irradiation of H-17 in the long range spin proton decoupling spectrum. These results led us to connect the partial structure A with the partial structure B. The ^1H NMR spectrum of a tetra acetyl dervative of FD-891 revealed that the four hydroxyl groups were located at C-7, C-10, C-21 and C-23, respectively.

The methyl signal (22-Me) at δ_H 0.80 showed cross peaks with the carbons for C-21 (δ_C 73.3), C-22 (δ_C 39.5) and C-23 (δ_C 78.2). A ^1H-^{13}C long range coupling to C-23 was observed from the doublet methyl (C-24) at δ_H 0.90, which in turn coupled to C-24 (δ_C 35.9) and C-25 (δ_C 82.5). Furthermore, the signals for C-24 and C-25 showed correlation peaks with the methyl (25-Me) at δ_H 1.20. A cross peak from the OMe at δ_H 3.25 to the signal for C-25 led to the location of the OMe substituent at C-25. By the HMBC spectrum were thus unambiguously confirmed and combined the fragments (5), (6) and (7) with each other as shown in Fig. 3 (the partial structure C). The HMBC technique was a very useful method for combination with the partial structures of FD-891. However, further information on the connectivities could not be obtained by the HMBC technique.

To get more information and determine relayed connectivities, the Homonuclear Hartmann-Hahn (HOHAHA)[8,9] experiment was applied. The HOHAHA method faciliates determination of the relayed connectivities of protonated functionalities by propagation of magnetization one after another.

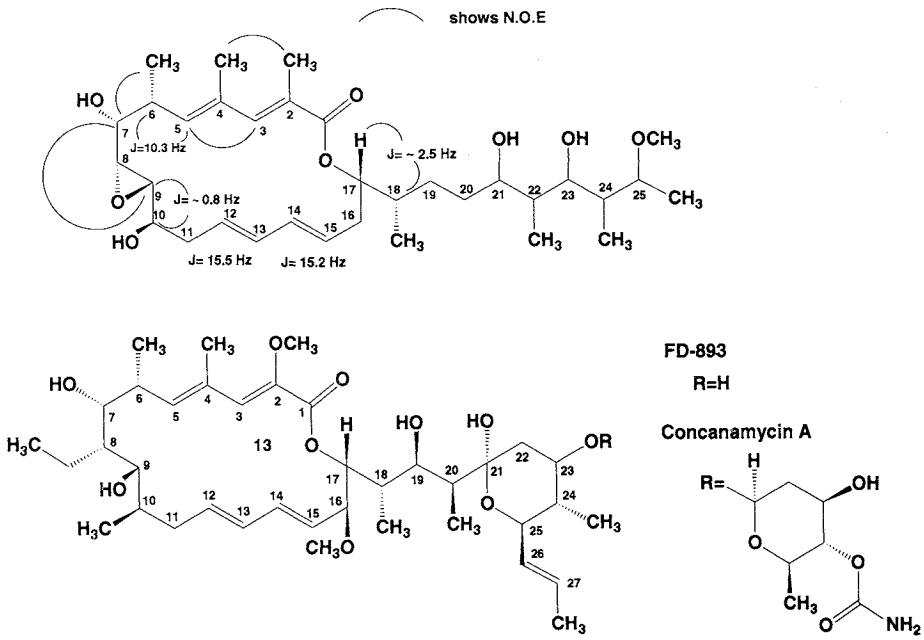
While refering to the ^1H-^1H COSY spectrum, respective cross peaks from H-21 at δ_H 3.88 and H-17 at δ_H 4.87 in the 2D HOHAHA spectrum allowed us to combine the methylene H-19A and -19B at δ_H 1.95 and 2.25 with the methylene H-20A and -20B at δ_H 2.15 and 2.30. The 2D HOHAHA spectrum made up connection with the partial structures B and C.

The remaining two olefinic functions at H-13 and H-14 were difficult to unequivocally distinguish and get a relation between them through the NMR methods because of their nearly identical chemical shifts. However, they were straightfowardly connected by taking into consideration the unsaturated degrees of FD-891 and the connectivities of the other functionalities. Thus, the structure of FD-891 was deduced as shown in Fig. 1.

Determination of the stereochemistry about the double bonds present in FD-891 was made by measurements of the NOEs and the $^3J_{H-H}$ coupling constants. The NOEs were observed between H-3 and H-5, 4-Me and 6-Me, indicating that the geometries of two double bonds from C-2 to C-5 were all *trans*. Because two pairs of coupled olefinic protons (H-12 and H-13, H-14 and H-15) had nearly identical chemical shifts, the coupling constants for these protons were measured from the 1H-detected ^1H-^{13}C shift correlation spectrum (HMQC)[10], without ^{13}C decoupling ^1H data aquisition. This experiment showed coupling constant values between H-12 and H-13, H-14 and H-15 to be 15.5 and 15.2 Hz, respectively, which revealed that the geometries of these two double bonds were all *trans*.

We have isolated not only FD-891 and FD-892 but also concanamycin A and its congeners FD-893, 894[11,12] from the fermentation broth of the same producing strain *Streptomyces graminofaciens* A-8890. Known biosynthetic studies on macrolide antibiotics such as erythromycin[13] suggested that these 18-membered antibiotics may be produced through functions of the same multi-enzymes. From a biosynthetic view point, the stereochemistry of the macrolide ring of FD-891 might be the same as that of concanamycin A. The absolute stereochemistry of concanamycin A[14] was determined by an X-ray analysis of its crystals

Fig. 4. Stereochemistry of FD-891.



as shown in Fig. 4. Therefore, we examined stereochemistry on the macrolide ring of FD-891 by comparison with concanamycin A.

H-5 coupled to H-6 in FD-891 by *ca.* 10.3 Hz of identical coupling constant value with that of concanamycin A. Similarly in concanamycin A, NOEs between 6-Me and H-7, H-6 and H-7 were observed in the NOESY spectrum of FD-891. Furthermore H-7 showed a cross peak with H-10 in the NOESY spectrum of FD-891. $^{3}J_{H-H} = \sim 2.5$ Hz between H-8 and H-9 in the epoxy ring indicated that their stereochemical relationships was *trans*. The vicinal coupling constant value (~ 0.8 Hz) between H-9 and H-10 suggested a torsion angle near 90°. As shown in Fig. 4. these results indicated that 6-Me, 7-OH and H-9 had α configurations whereas H-8 and 10-OH were β, in the same of FD-891as in concanamycin A. The coupling constant between H-17 and H-18 was very small (~ 2.5 Hz) and it was difficult to trace a correlation peak of H-17 and H-18 in the ^{1}H-^{1}H COSY spectrum of FD-891 as well as that of concanamycin A. It was implicated that the stereochemistry at C-17 of FD-891 was identical with that of concanamycin A. To determine the stereochemistry of 1,3 diol at C-21 and C-23 by application of analysis of ^{13}C NMR of acetonide derivatives[15], we tried to synthesize an acetonide derivative of FD-891, however, we failed to obtain one because of decomposition of FD-891 by *p*-toluene sulfonic acid wich was used as catalyst. We will isolate a large quantity of FD-892 (*vide infra*) and synthesize its acetonide derivative in the near future to determine the stereochemistry at C-21 and C-23. The other stereochemistry remained to be assigned, and is now under study.

Structure of FD-892

The physico-chemical properties are shown in Table 1. The UV spectrum of FD-892 was very similar to that of FD-892, suggesting that its chromophore was the same as that of FD-891. Also, the IR spectrum of FD-892 showed the existence of a ester carbonyl at 1705 cm^{-1} and a hydroxy group at 3400 cm^{-1}.

FD-892 had a molecular formula of $C_{32}H_{52}O_6$ established by the observation of its molecular ion at *m/z* 532.3759 (calcd. 532.3764 for $C_{32}H_{52}O_6$) in the HREI-MS spectrum in combination with its NMR spectra. The molecular formula of FD-892 gave seven degree of unsaturation. The appearance of resonances for ten sp^2 carbons and a carbonyl group in the ^{13}C NMR spectrum indicated that 5 degrees of unsaturation were attributed to the presence of five double bonds and the last degree could be satisfied by assignment of one ring. By its ^1H and DEPT spectrum were only 49 hydrogens identified, revealing the presence of three hydroxyl groups in the molecule.

Since the physico-chemical properties of FD-892 were similar to those of FD-891, the structural assignment was initiated by comparison of the NMR spectra of FD-892 with those of FD-891.

The ^1H and ^{13}C NMR data are listed in Table 2.

The ^{13}C NMR spectrum of FD-892 showed a similar spectral pattern to that of FD-891 except for the disappearance of the signals for C-8 at δ_C 55.1, C-9 at δ_C 56.0, OCH_3 at δ_C 56.0 and C-10 at δ_C 71.0, the appearance of the line at δ_C 29.9 and one pair of the protonated olefinic carbons at δ_C 125.5 and 133.2 and the downfield shift of the signal corresponding to C-25 from δ_C 72.8 to δ_C 82.2. These spectral data indicated that the double bond was formed at C-8 and C-9 in place of an epoxide ring and that a hydroxyl group at C-10 and a OCH_3 group at C-25 were lost in the molecule of FD-892. In the ^1H NMR spectrum of FD-892 a OCH_3 signal at δ_H 3.34 and two proton signals at δ_H 3.15 and 3.25 due to an epoxide ring disappeared and the increase of 2H of integeration value at the olefinic region from δ_H 5.30 to δ_H 5.90 was observed.

To unambiguously elucidate the structure, the NMR spectra was analyzed in detail. The HMBC experiment from the methyl signals at C-22, C-24 and C-25 made it possible for the structure from C-21 to C-25 to be deduced, which indicated the replacement of the OMe by a hydroxyl group at C-25. The upfieldshift by 9.8 ppm at C-25 was explained by well-known methylation shift.

By the HMBC method the same structure from C-1 to C-7 was elucidated as in FD-891. The signal due to H-7 at δ_H 3.85 showed a cross peak of the line at δ_H 5.34 (H-8), corresponding to the carbon at δ_C 125.5, which in turn coupled to the olefinic proton at δ_H 5.78 (H-9). The signal at H-9 matched with the line at δ_C 133.2 in the ^1H-^{13}C COSY spectrum. Furthermore, the resonance of H-9 coupled to an unequivalent methylene protons at δ_H 1.95 and 2.01 (H-10A and H-10B), which made up cross peaks of the line at δ_C 29.9 (C-10) in the ^1H-^{13}C spectrum. These spectral analysis led to the conclusion that an epoxide ring at C-8 and C-9 and a hydroxyl group at C-10 were lost and a duoble bond at C-8 and C-9 was formed in FD-892.

The measurement of 16.0 Hz of the coupling constant between H-8 and H-9 by the HMQC spectrum showed that the geometry of the double bond was *E*. The geometry of the other double bonds was the same as in FD-891. Both the ^1H and ^{13}C NMR spectra from C-10 to C-24 of FD-892 were basically the same as those of FD-891, indicating that both FD-891 and FD-892 had the same structure from C-10 to C-24. Thus, the structure of FD-892 was elucidated as shown in Fig. 1.

Experimental

General

IR spectra were recorded on a Perkin-Elmer 1760 FI-IR spectrophotometer. UV spectra were measured on a Hitachi 220A spectrophotometer. EI-MS and FAB-MS spectra were obtained with a JEOL JMX-SX 102 mass spectrometer. NMR spectra were measured on a JEOL JMN-GX 400 spectrometer at ambient

temperature at 400 MHz (^{1}H) and 100 MHz (^{13}C) using the solvent peaks as internal references downfield of TMS at 0 ppm.

A Tetra Acetyl Derivative of FD-891

15 mg of FD-891 was solved in 0.7 ml of pyridine. To this solution was added 0.5 ml of acetic anhydride and 3 mg of 2-methyl amino pyridine as a catalyst. The solution was stirred for 6 hours at room temperature. To the reaction mixture was added 20 ml of cold water and then 20 ml of ethyl acetate. The ethyl acetate layer was dried over Na_2SO_4 and concentrated *in vacuo* to give a solid material, which was subjected to LH-20 column chromatography developed with MeOH. The fractions containing a tetra acetyl derivative was concentrated *in vacuo* to obtain 10 mg of oily material. The mass and NMR spectral data of a tetra acetyl derivative of FD-891 thus obtained were as follows.

EI-MS *m/z* 746 (M^+), FAB-MS *m/z* 747 $(M+H)^+$, 769 $(M+Na)^+$ ^{1}H NMR (in $CDCl_3$): δ_H 7.32 (H-4), 5.53 (H-5), 3.14 (H-6), 5.30 (H-7), 3.23 (H-8), 3.15 (H-9), 5.38 (H-10), 2.26 (H-11), 5.66 (H-12), 5.55 (H-13), 5.60 (H-14), 5.77 (H-15), 2.20 (H-16A), 2.29 (H-16B), 4.87 (H-17), 1.84 (H-18), 1.95 (H-19A), 2.25 (H-19B), 2.15 (H-20A), 2.30 (H-20B), 4.63 (H-21), 1.80 (H-22), 5.21 (H-23), 1.61 (H-24), 3.60 (H-25), 3.34 ($-OCH_3$), 2.10 (2-CH_3), 2.03 (4-CH_3), 1.17 (6-CH_3), 0.89 (18-CH_3), 0.80 (22-CH_3), 0.90 (24-CH_3), 1.20 (25-CH_3), 2.10 ($-OCOCH_3$), 2.11 ($-OCOCH_3$), 2.31 ($-OCOCH_3$), 2.33 ($-OCOCH_3$)

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