STUDIES ON NEW PHOSPHATE ESTER ANTIFUNGAL ANTIBIOTICS PHOSLACTOMYCINS

II. STRUCTURE ELUCIDATION OF PHOSLACTOMYCINS A TO F

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Phoslactomycins A to F are new antifungal antibiotics produced by *Streptomyces nigrescens* SC-273. On the basis of physico-chemical properties and NMR studies, their structures have been determined as shown in Fig. 6. They are characterized by possessing α,β-unsaturated δ-lactone, phosphate ester, conjugated diene and cyclohexane ring moieties. The structural difference between them is ascribed to a substituent bound to the cyclohexane ring.

New antifungal antibiotics phoslactomycins A to F were obtained from the culture broth of *Streptomyces nigrescens* SC-273, a soil isolate. Taxonomy of the producing strain, fermentation, purification and biological activities of phoslactomycins have been reported in the preceding paper[1]. This paper deals with the physico-chemical properties and structure elucidation of phoslactomycins A to F.

Materials and Methods

Instruments

The UV and IR spectra were recorded on a Shimadzu UV-240 and a Digilab FTS-15C Fourier transformation (FT)-IR spectrometer, respectively. Optical rotations were measured on a Jasco DIP-140. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and ^{31}P NMR (162 MHz) spectra were obtained on a Jeol GX-400 FT NMR spectrometer. Fast atom bombardment mass spectra (FAB-MS) were measured with a Jeol JMS-DX303 spectrometer using glycerol as the matrix. MP's were determined with a Yanaco MP-S3 micro melting point apparatus and are uncorrected.

Alkaline Hydrolysis of Phoslactomycins and Identification of Aliphatic Carboxylic Acids

A solution of 1 mg phoslactomycin E in 1 ml of 1 N KOH was kept at 37°C for 5 hours. The solution was extracted with 2 ml of $CHCl_3$ under acidic conditions. The organic layer was concentrated *in vacuo* and analyzed by GC-MS. Identification of aliphatic carboxylic acids in the hydrolysate was made by comparison with an authentic sample of cyclohexanecarboxylic acid. GC was carried out with a Shimadzu GC-4B at 140°C on a column packed with 10% SP-1000+1% H_3PO_4/Chromosorb W (AWDMCS).

Phoslactomycin F (100 μg) was hydrolyzed in the same way as phoslactomycin E and analyzed by GC. The aliphatic acid in the hydrolysate was identified by comparison with authentic samples (3-methylhexanoic acid and 4-methylhexanoic acid). GC was carried out with a Shimadzu GC-4B at 140°C on a column packed with 10% SP-1200+1% H_3PO_4/Chromosorb W (AW). Retention times of 3-methylhexanoic acid and 4-methylhexanoic acid were 15.3 and 18.1 minutes, respectively.

Results

Physico-chemical Properties

Physico-chemical properties of phoslactomycins A to F are summarized in Table 1. Since the

Table 1. Physico-chemical properties of phoslactomycins A to F.

	A	B	C	D	E	F
MP (°C)	165~168	161~163	165~168	*	168~171	*
Molecular formula	$C_{29}H_{46}O_{10}NP$	$C_{25}H_{40}O_8NP$	$C_{30}H_{48}O_{10}NP$	$C_{31}H_{50}O_{10}NP$	$C_{32}H_{50}O_{10}NP$	$C_{32}H_{52}O_{10}NP$
FAB-MS (*m/z*)	600 $(M+H)^+$,	514 $(M+H)^+$,	614 $(M+H)^+$,	672 $(M-H+2Na)^+$,	640 $(M+H)^+$,	624 $(M+H-H_2O)^+$,
	622 $(M+Na)^+$,	496 $(M+H-H_2O)^+$,	636 $(M+Na)^+$,	626 $(M-H)^-$	662 $(M+Na)^+$,	642 $(M+H)^+$,
	598 $(M-H)^-$	512 $(M-H)^-$	612 $(M-H)^-$		638 $(M-H)^-$	640 $(M-H)^-$
HRFAB-MS (*m/z*)	$(M-H+2Na)^+$	$(M+H-H_2O)^+$	$(M-H+2Na)^+$	*	$(M+H)^+$	*
Found:	644.2573	496.2461	658.2749		640.3275	
Calcd:	644.2577	496.2464	658.2734		640.3251	
UV λ_{max}^{MeOH} nm	233 (ε 24,600)	233	233	233	233	233
$[\alpha]_D^{25}$ (MeOH)	+61° (*c* 0.2)	+23° (*c* 0.1)	+40° (*c* 0.1)	+33° (*c* 0.02)	+61° (*c* 0.2)	+37° (*c* 0.02)
Rf value on TLC[a]	0.77	0.69	0.58	0.42	0.39	0.35
^{31}P NMR[b] δ	3.95, d, J=9.5 Hz	2.59, d, J=9.5 Hz	3.27, d, J=10.0 Hz	*	2.85, d, J=9.5 Hz	*

Appearance: Colorless powder. Solubility: Soluble in MeOH, slightly soluble in H_2O, insoluble in $CHCl_3$.
Color reactions: Positive to ninhydrin (purple), ammonium molybdate - perchloric acid (blue), negative to anisaldehyde.
[a] 80% aq MeOH; RP-18 (Merck). [b] δ from H_3PO_4 in CD_3OD.
* Data were not obtained due to lack of samples.

Fig. 1. FT-IR spectrum of phoslactomycin E (MeOH).

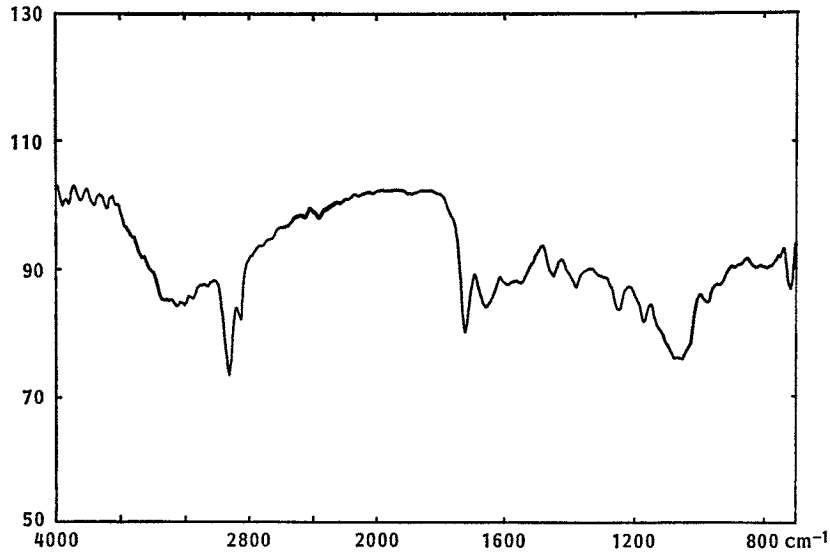


Table 2. ^{13}C NMR spectral data for phoslactomycins A, B, C and E.

Carbon	A	B	C	E
C-1	166.4 s[a]	166.4 s	166.4 s	166.4 s
C-2	121.0 d	121.0 d	121.0 d	121.0 d
C-3	152.7 d	152.7 d	152.7 d	152.7 d
C-4	40.6 d	40.5 d	40.6 d	40.5 d
C-5	82.3 d	82.3 d	82.4 d	82.3 d
C-6	127.6 d	127.5 d	127.4 d	127.6 d
C-7	137.4 d	137.7 d	137.9 d	137.5 d
C-8	77.8 s	78.1 s	78.2 s	77.9 s
C-9	78.3 d	78.1 d	77.7 d	78.2 d
C-10	40.6 t	40.5 t	40.6 t	40.5 t
C-11	64.7 d	64.6 d	64.5 d	64.6 d
C-12	135.2 d	134.5 d	135.4 d	135.2 d
C-13	124.3 d	124.5 d	124.1 d	124.2 d
C-14	123.7 d	122.9 d	123.9 d	123.7 d
C-15	138.2 d	140.1 d	138.2 d	138.1 d
C-16	36.1 d	37.6 d	36.1 d	36.1 d
C-17	39.3 t	34.3 t	39.4 t	39.3 t
C-18	73.8 d	26.9 t	73.9 d	73.7 d
C-19	32.3 t	27.0 t	32.4 t	32.3 t
C-20	24.6 t	26.9 t	24.6 t	24.6 t
C-21	33.0 t	34.3 t	33.1 t	33.0 t
C-22	22.7 t	22.7 t	22.6 t	22.7 t
C-23	11.3 q	11.3 q	11.3 q	11.4 q
C-24	34.1 t	33.6 t	33.1 t	33.9 t
C-25	37.1 t	37.2 t	37.3 t	37.1 t
C-1′	178.3 s		174.3 s	177.3 s
C-2′	35.3 d		44.7 t	44.5 d
C-3′	19.3 q		27.0 d	30.1 t
C-4′	19.3 q		22.6 q	26.4 t
C-5′			22.6 q	26.9 t
C-6′				26.4 t
C-7′				30.1 t

Chemical shifts in ppm downfield from internal TMS (0 ppm) in CD_3OD.

[a] Multiplicity.

UV and ^{1}H NMR spectra of phoslactomycins A to F resembled each other very closely, they were reasonably assumed to be structural analogs.

Phoslactomycins were shown to contain an amino moiety and phosphorus by their positive color reactions with ninhydrin and ammonium molybdate-perchloric acid, respectively. The phosphorus in phoslactomycin E proved to exist as a phosphate ester based on its ^{31}P NMR data (2.85 ppm, d, $^{3}J_{P\text{-}O\text{-}C\text{-}H}=9.5$ Hz). The IR spectrum of phoslactomycin E (Fig. 1) showed major absorptions at 1724, 1076 and 1250 cm^{-1} signifying the presence of α,β-unsaturated δ-lactone, P-O, and P=O, respectively. UV spectra (absorption at 233 nm) suggested the presence of an α,β-unsaturated δ-lactone.

Since the major component of phoslactomycins was phoslactomycin E, its structure was elucidated first. The structure analyses of the remaining components were then made based on comparison with the established structure of phoslactomycin E.

Structure Elucidation of Phoslactomycin E

The molecular formula of phoslactomycin E was determined to be $C_{32}H_{50}O_{10}NP$ by analysis of high-resolution (HR)FAB-MS, ^{1}H, ^{13}C and ^{31}P NMR data (Tables 1, 2 and 3).

The ^{13}C NMR spectrum (Table 2) revealed the presence of 32 carbon signals, which were attributed to one methyl carbon, 13 methylene carbons, three methine carbons, four oxymethine carbons, one quaternary oxycarbon, 8 olefinic carbons, and two ester carbonyls by distortionless enhancement by polarization transfer (DEPT) experiments.

The ^{1}H NMR spectrum of phoslactomycin E (Fig. 2) showed 44 non-exchangeable proton signals (Table 3). Since total number of protons in phoslactomycin E is 50, 6 protons are ascribed to exchangeable protons.

Analysis of 2D correlation spectroscopy (COSY) spectrum[2] revealed the presence of the following partial structures; Unit I (Fig. 3), Unit II (Fig. 5B), Unit III (Fig. 5A) and Unit IV (Fig. 5C). Assignment of the carbon signals was based on ^{13}C-^{1}H shift correlation spectrum[2]. The remaining

Fig. 2. ^{1}H NMR spectrum of phoslactomycin E (400 MHz, CD_3OD).

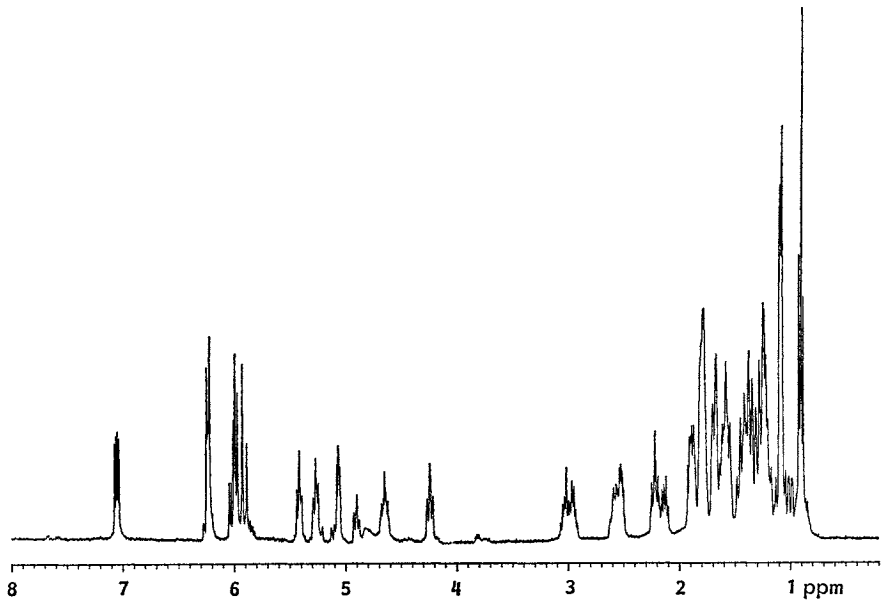


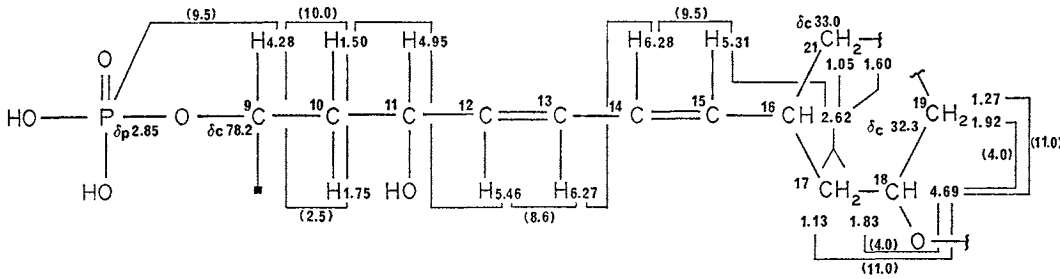
Table 3. ^{1}H NMR spectral data for phoslactomycins A to F.

Proton	A	B	C	D	E	F
2-H	6.01[a] dd[b]	6.02 dd	6.01 dd	6.01 dd	6.02 dd	6.01 dd
	J=1.0, 9.5[c]	J=1.1, 9.5	J=1.0, 9.5	J=1.0, 9.5	J=1.2, 9.5	J=1.1, 9.5
3-H	7.08 dd	7.09 dd	7.08 dd	7.09 dd	7.09 dd	7.09 dd
	J=4.8, 9.5	J=5.0, 9.5	J=5.0, 9.5	J=4.8, 9.5	J=5.1, 9.5	J=5.0, 9.5
4-H	2.55 m	2.56 m	2.55 m	2.55 m	2.56 m	2.55 m
5-H	5.10 dd	5.10 dd	5.09 dd	5.08 dd	5.10 dd	5.09 dd
	J=5.2, 6.2	J=5.1, 6.5	J=4.9, 6.8	J=5.0, 6.7	J=5.1, 6.5	J=5.0, 6.5
6-H	6.06 dd	6.09 dd	6.07 dd	6.13 dd	6.07 dd	6.12 dd
	J=6.2, 15.1	J=6.5, 15.2	J=6.8, 15.8	J=6.7, 15.1	J=6.5, 15.1	J=6.5, 15.5
7-H	5.93 dd	5.92 dd	5.93 d	5.88 d	5.93 dd	5.89 dd
	J=15.1, 1.0	J=0.8, 15.2	J=15.8	J=15.1	J=1.0, 15.1	J=1.0, 15.5
9-H	4.28 ddd	4.27 ddd	4.28 ddd	4.24 ddd	4.28 ddd	4.25 ddd
	J=2.0, 9.5, 9.5	J=2.0, 9.5, 9.5	J=1.8, 10.0, 10.0	J=2.0, 10.0, 10.0	J=2.5, 9.5, 10.0	J=2.4, 10.5, 10.5
10-H_a	+	1.50	+	1.48	1.50	+
10-H_b	+	1.67	+	+	1.75	+
11-H	4.95	4.98	4.95	5.02	4.95	5.00
12-H	5.45	5.41	5.45	5.45	5.46	5.45
13-H	6.27	6.24	6.27	6.27	6.27	6.27
14-H	6.28	6.25	6.28	6.28	6.28	6.28
15-H	5.31	5.32	5.30	5.28	5.31	5.29
16-H	2.62	2.46	2.62	2.62	2.62	2.61
17-H_{ax}	+	+	+	+	1.13	+
17-H_{eq}	+	+	+	+	1.83	+
18-H	4.69 tt	+	4.72 tt	4.71 tt	4.69 tt	4.71 tt
	J=4.5, 10.0		J=4.2, 10.0	J=4.3, 11.0	J=4.0, 11.0	J=4.1, 11.0

19-H_{ax}	+	+	+	+	1.27	+
19-H_{eq}	+	+	+	+	1.92	+
20-H_{ax}	+	+	+	+	1.49	+
20-H_{eq}	+	+	+	+	1.81	+
21-H_{ax}	+	+	+	+	1.05	+
21-H_{eq}	+	+	+	+	1.60	+
22-H_a	+	1.52	1.49	+	1.45	+
22-H_b	+	1.62	1.67	+	1.62	+
23-H	0.95 t	0.96 t	0.95 t	0.95 t	0.95 t	0.95 t
	J=7.5	J=7.0	J=7.0	J=7.2	J=7.0	J=7.0
24-H_a	+	1.85 m	1.91	1.92	1.92	1.85
24-H_b	2.18 m	2.21 m	2.21 m	2.25 m	2.20 m	2.25
25-H	3.03 m	3.04 m	3.03 m	3.05 m	3.04 m	3.05 m
2′-H	2.49 m		2.14 d	2.27 t	2.26 tt	2.27
	J=6.5		J=7.0	J=7.5	J=3.7, 10.9	
3′-H	1.11 d		2.04 m	1.46	ax 1.32	+
	J=6.5				eq 1.82	
4′-H	1.11 d		0.93 d	1.52	ax 1.27	+
	J=6.5		J=6.9		eq 1.67	
5′-H			0.93 d	0.89 d	ax 1.17	+
			J=6.9	J=6.5	eq 1.61	
6′-H				0.89 d	ax 1.27	0.88 t
				J=6.5	eq 1.67	J=7.0
7′-H					ax 1.32	0.89 d
					eq 1.82	J=6.5

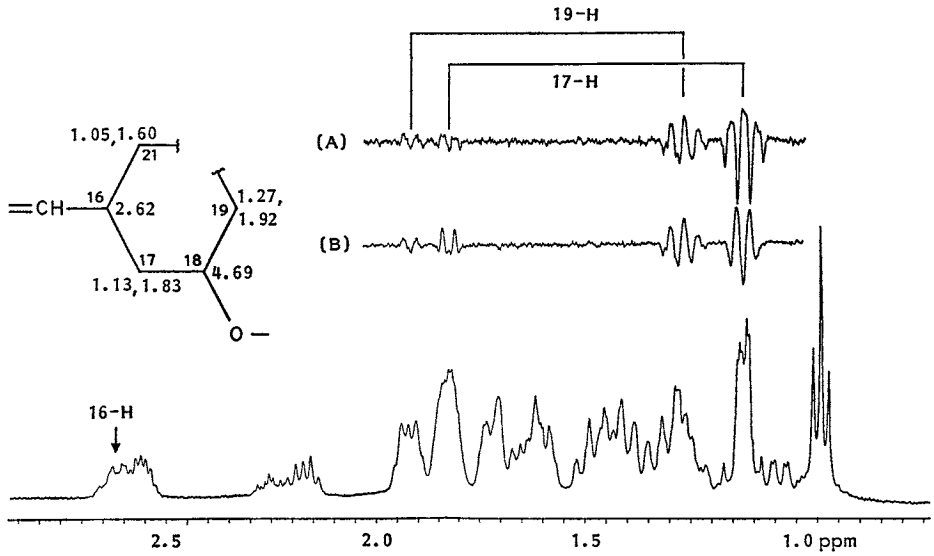
[a] Chemical shifts in ppm from internal TMS (0 ppm) in CD_3OD. [b] Multiplicity. [c] J in Hz. +: Assignment could not be made due to severe overlapping of signals.

Fig. 3. Structure of Unit I.



δ_H in CD_3OD (J in Hz)

Fig. 4. Decoupling difference spectra.



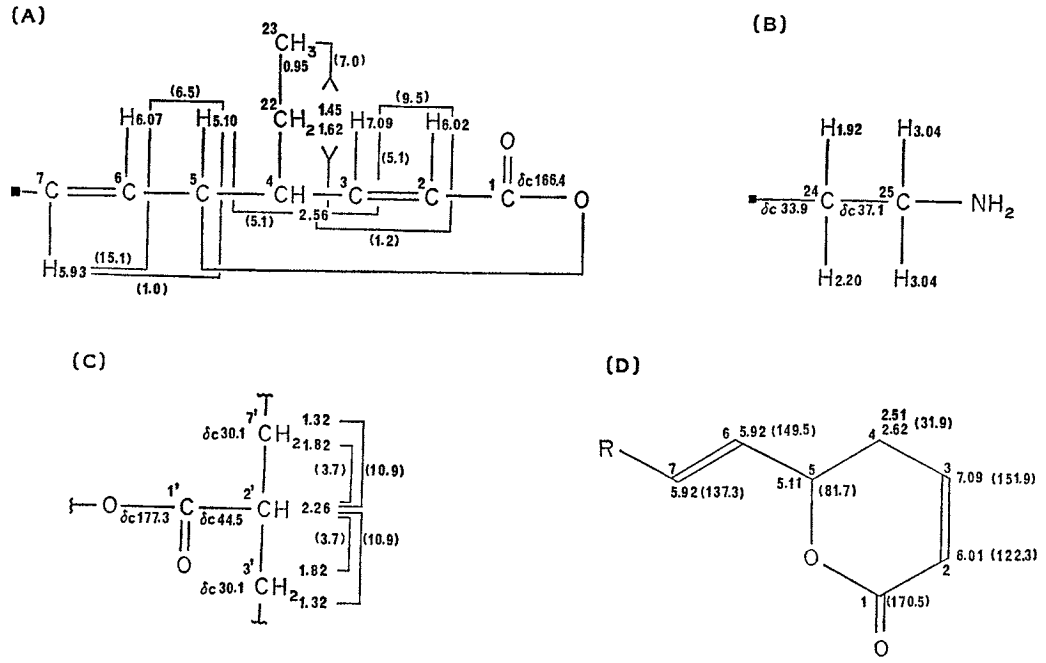
(A) Subtracting non-decoupled spectrum from decoupled spectrum irradiated at 18-H (4.69 ppm).
(B) Subtracting decoupled spectrum irradiated at 16-H from decoupled spectrum simultaneously irradiated at 18-H and 16-H (2.62 ppm).

carbons not appearing in these Units are one quaternary oxycarbon (77.9 ppm) and 4 methylenes with almost identical chemical shifts (24.6, 26.4×2 and 26.9 ppm).

In Unit I (Fig. 3), the spin system from 9-H to 16-H was established straightforwardly by analysis of the 2D COSY spectrum. The sequence from 16-H to 19-H was not elucidated unambiguously due to overlapping of proton signals at 1.1~1.9 ppm. This spin system was clarified by decoupling difference spectra (DDS). DDS obtained by irradiating 18-H (4.69 ppm) revealed that C-18 was linked to two none equivalent methylenes (Fig. 4A). One was 19-H at 1.27 and 1.92 ppm, and the other was 17-H at 1.13 and 1.83 ppm. DDS with additional irradiation at 16-H (2.62 ppm) revealed the collapse of the 17-H proton signals only at 1.13 and 1.83 ppm (Fig. 4B). These results proved the network from C-16 to C-19. Similarly, connectivity between C-16 and C-21 was established by DDS irradiating at 16-H.

Although the further extensions of the spin systems from 19-H and 21-H were not feasible due to

Fig. 5. Structures of Units II (B), III (A), IV (C) and relevant partial structure of a model compound (D)[5].



severe overlap of proton signals, the ^{13}C NMR chemical shifts of C-19 and C-21 (32.3 and 33.0 ppm) and the signal shapes of 17-H and 19-H (see Fig. 4) and 18-H protons suggested that C-19 and C-21 were linked to each other *via* one of the four unassigned methylene carbons (*vide supra*) to construct a cyclohexane ring. The signal shape of the oxymethine 18-H (4.69 ppm, tt, J=4.0 and 11.0 Hz, C-18 73.7 ppm) suggested it to be an axial proton in a cyclohexane ring. Taking into account the different alkyl substituent effects on the cyclohexane ring, the ^{13}C chemical shifts of C-16 to C-19 and C-21 and one of the methylene carbons (24.6, 26.4×2 and 26.9 ppm) to be assigned to C-20 can be compared with those of *cis*-3-methylcyclohexanol (C-1 70.5, C-2 44.6, C-3 31.5, C-4 35.3, C-5 24.3 and C-6 34.2)[3].

^{31}P NMR data (2.85 ppm, d, $^3J_{P\text{-}O\text{-}C\text{-}H}$=9.5 Hz), IR absorption (1250 and 1076 cm^{-1}) and the positive color reaction to ammonium molybdate-perchloric acid indicated the presence of a phosphate ester in phoslactomycin E. The location of this moiety was determined to be at C-9 because the ^{31}P signal collapsed to a singlet when the 9-H methine proton was selectively irradiated. The NMR chemical shifts of 9-H and C-9 (4.28 and 78.2 ppm, respectively) and the splitting pattern of 9-H suggested that C-9 carbon was attached to a quaternary carbon.

The configuration of the conjugated diene system was established as Z, Z on the basis of the coupling constants ($J_{12,13}$=8.6 Hz and $J_{14,15}$=9.5 Hz) measured by spin-decoupling experiments.

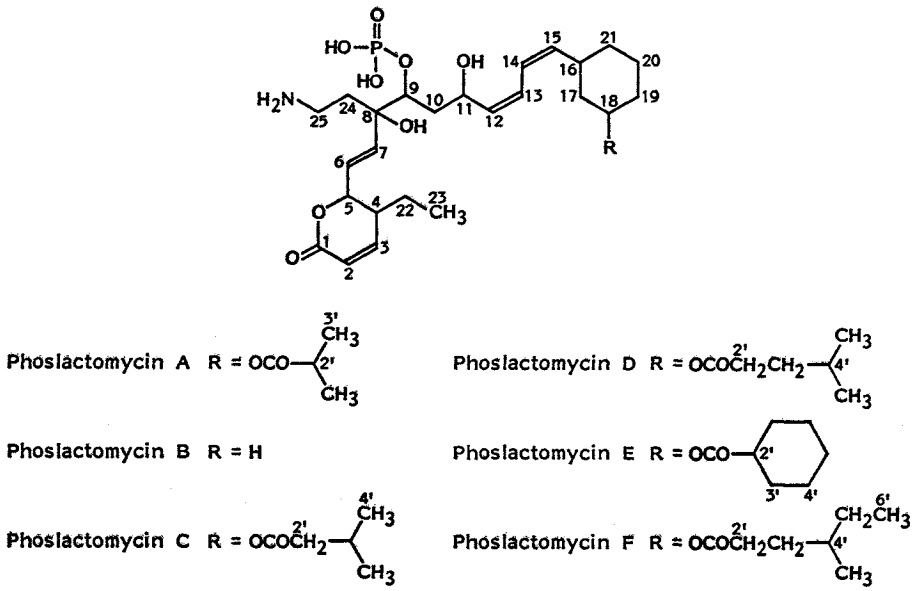
Spin system in Unit II (Fig. 5B) consisted of only two methylenes (24-H and 25-H) with an amino substituent on C-25 as revealed by the ^1H and ^{13}C NMR chemical shifts (25-H, 3.04 ppm and C-25, 37.1 ppm) and by a positive reaction (purple) with ninhydrin. Linkage of C-24 to a quaternary carbon was indicated by the ^1H and ^{13}C NMR chemical shifts (1.92, 2.20 and 33.9 ppm) of 24-position and splitting pattern of 24-H.

In Unit III (Fig. 5A), the spin system from 2-H to 7-H was established by analyzing the 2D COSY spectrum. The chemical shifts of 2-H and 3-H (6.02 and 7.09 ppm) indicated that an ester carbonyl (C-1 at 166.4 ppm) was attached at C-2. This linkage was confirmed by the long range coupling (between C-1 and 2-H and 3-H in heteronuclear multiple bond connectivity (HMBC) spectrum[4]) and IR absorption at 1724 cm^{-1}. The chemical shift of 5-H (5.10 ppm) indicated the formation of a lactone ring including C-1 to C-5. This value is almost identical with that of the corresponding proton (5-H, 5.11 ppm) in the 6-vinyl-5,6-dihydropyran-2-one portion of CI-920[5] (Fig. 5D). The presence of the δ-lactone ring in phoslactomycin E was supported by the similar ^{13}C chemical shifts of C-1 to C-7 between the model compound just mentioned and phoslactomycin E. The stereochemistry of the olefinic bond at C-6 was determined to be *E* ($J_{6,7}$=15.1 Hz). The linkage of C-7 to a quaternary carbon was deduced by analysis of the HMBC spectrum described below.

In Unit IV (Fig. 5C), extensions of the spin systems from 3′-H and 7′-H were obstructed by severe overlapping of proton signals; construction of a cyclohexane ring, however, was suggested by the shape of the 2′-H signal appearing as a triplet of triplets (J=3.7 and 10.9 Hz) which clearly indicated 2′-H to be an axial proton in a cyclohexane ring. The ^{1}H and ^{13}C NMR chemical shifts of the 2′-position (2.26 and 44.5 ppm) suggested its linkage to a carbonyl carbon (C-1′ at 177.3 ppm). In agreement with this conclusion, the ^{13}C chemical shifts of C-1′, C-2′, C-3′ (30.1 ppm), C-7′ (30.1 ppm) and three remaining methylene carbons (26.4×2 and 26.9 ppm) can be compared with those of cyclohexanecarboxylic acid[6]. The presence of the cyclohexanecarbonyl moiety was also confirmed by detecting cyclohexanecarboxylic acid in the alkaline hydrolysate of phoslactomycin E by GC-MS. The binding position of the ester linkage was determined using the acyl shift of 18-H described below.

Since the only carbon remaining to be analyzed at this point was a quaternary oxycarbon (C-8), the established Units I, II and III must be combined through this quaternary carbon. These relationships were corroborated by HMBC spectral data. Observation of long range coupling between C-8 and 9-H, 24-H and 25-H in the HMBC spectrum proved this linkage. Furthermore, C-8 had long

Fig. 6. Structures of phoslactomycins.



range coupling with 6-H and 7-H (Unit III) in the HMBC spectrum. The binding position of the cyclohexanecarbonyl moiety was determined to be at C-18 based on the downfield shift of 18-H (4.69 ppm) as compared with the corresponding proton (3.60 ppm) in *cis*-3-methylcyclohexanol[7]. Thus, the whole planar structure of phoslactomycin E was established as shown in Fig. 6.

Structure Elucidation of Phoslactomycins A, C, D and F

As described above, phoslactomycins A, C, D and F were judged to be structural analogs of phoslactomycin E by comparison of their UV and ^{1}H NMR spectra.

Analysis of HRFAB-MS, ^{1}H, ^{13}C and ^{31}P NMR data (Tables 1, 2 and 3) indicated that the molecular formulae of phoslactomycins A and C were $C_{29}H_{46}O_{10}NP$ and $C_{30}H_{48}O_{10}NP$, respectively. Since phoslactomycins D and F were minor components of phoslactomycins, HRFAB-MS, ^{13}C and ^{31}P NMR spectra were not obtained due to paucity of samples. By the analysis of FAB-MS and ^{1}H NMR data (Tables 1 and 3), the molecular formulae of phoslactomycins D and F were determined to be $C_{31}H_{50}O_{10}NP$ and $C_{32}H_{52}O_{10}NP$, respectively.

Analyses of 2D COSY spectra revealed that phoslactomycins A, C, D and F had the same spin system from 2-H to 25-H as that of phoslactomycin E. ^{13}C NMR data supported the presence of this common structural unit in phoslactomycins A and C (Table 2). Therefore, the structural difference between these compounds and phoslactomycin E is only the acyl moiety bound to C-18. Structure analysis of the acyl moiety resulted in the whole structure elucidation as follows.

By analyses of 2D COSY spectra, the acyl moieties in phoslactomycins A, C and D were determined to be isobutyryl (2′-H methine at 2.49 ppm and 3′-H and 4′-H methyls at 1.11 ppm), isovaleryl (2′-H methylene at 2.14 ppm, 3′-H methine at 2.04 ppm and 4′-H and 5′-H methyls at 0.93 ppm) and 4-methylpentanoyl (2′-H methylene at 2.27 ppm, 3′-H methylene at 1.46 ppm, 4′-H methine at 1.52 ppm and 5′-H and 6′-H methyls at 0.89 ppm) moieties, respectively. The whole structures of phoslactomycins A, C and D thus determined are illustrated in Fig. 6.

In the structure elucidation of phoslactomycin F, the acyl moiety could not be clearly determined by 2D COSY due to overlap of proton signals. Two methyls (0.89 ppm, d and 0.88 ppm, t) and one methylene (2.27 ppm) were observed in the ^{1}H NMR spectrum. These spectral data suggested that the substituent was a 3-methylhexanoyl or a 4-methylhexanoyl moiety. Alkaline hydrolysis of phoslactomycin F followed by GC analysis detected the presence of 4-methylhexanoic acid in phoslactomycin F. Thus, the whole structure of phoslactomycin F was determined as shown in Fig. 6.

Structure Elucidation of Phoslactomycin B

The structure of phoslactomycin B was also assumed to be analogous to phoslactomycin E by comparison of their UV and ^{1}H NMR spectra. HRFAB-MS data (Table 1) established the molecular formula of phoslactomycin B as $C_{25}H_{40}O_8NP$. ^{13}C and ^{1}H NMR data are summarized in Tables 2 and 3, respectively. Comparison of the ^{13}C NMR spectral data between phoslactomycins B and E clearly showed that phoslactomycin B had the same carbon skeleton (from C-1 to C-25) as phoslactomycin E with only exception being replacement of the oxymethine group (C-18, 73.7 ppm) in phoslactomycin E by a methylene carbon phoslactomycin in B (26.9 ppm). In agreement with this structural modification, C-17 and C-19 moved to upfield, and C-16 and C-20 moved to downfield due to the disappearance of β- and γ-effects, respectively. Assignment of the five methylene signals of the cyclohexane ring moiety was made based on the ^{13}C NMR data of cyclohexanecarboxylic acid[6]. Thus,

the structure of phoslactomycin B was determined as shown in Fig. 6.

Discussion

Chemical structures of phoslactomycins A to F were elucidated mainly by analyzing NMR spectral data. They have an unsaturated δ-lactone ring, a phosphate ester and a cyclohexyl residue in common. The cyclohexyl residue is rather unusual in natural products; it has been found in mycotrienin[8] and asukamycin[9]. As described in the preceding paper[1], the substituent on the cyclohexane ring is not important for their biological activities.

Among the known antibiotics, CI-920[5,10] is structurally similar to phoslactomycin, and phosphazomycin A[11] of unknown structure seems to be closely related to phoslactomycin. Although phoslactomycins are characterized by their high antifungal activities[1], CI-920 showed strong antitumor activity without showing antimicrobial activity. Main structural differences between them are as follows; phoslactomycin has an aminoethyl moiety at C-8 position instead of methyl group and a cyclohexyldienyl residue instead of a hydroxymethyltrienyl group. Therefore, the aminoethyl and cyclohexyldienyl moieties may be required for expression of antifungal activities of phoslactomycins. Further studies will uncover the interesting structure-biological relationships of phoslactomycins and CI-920.

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