TIACUMICINS, A NOVEL COMPLEX OF 18-MEMBERED MACROLIDES

II. ISOLATION AND STRUCTURE DETERMINATION

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A novel complex of Gram-positive antibiotics has been isolated from the fermentation broth and mycelium of *Dactylosporangium aurantiacum* subsp. *hamdenensis* subsp. nov. The structures of these six compounds were deduced employing UV, MS, IR, and extensive 1D and 2D homonuclear and heteronuclear NMR experiments. Each component contained a highly unsaturated 18-membered macrolide ring. Components differed from one another by minor structural variations in the macrolide ring and by the number and esterification pattern of glycosidically bound sugars.

In the course of screening microorganisms for the production of antibiotics a *Dactylosporangium* species was discovered which produced a novel complex of 18-membered macrolides. The companion paper[1] describes the taxonomy and fermentation of this organism and the biological activity of the individual antibiotics. This paper will describe the isolation of these components and the elucidation of their structures.

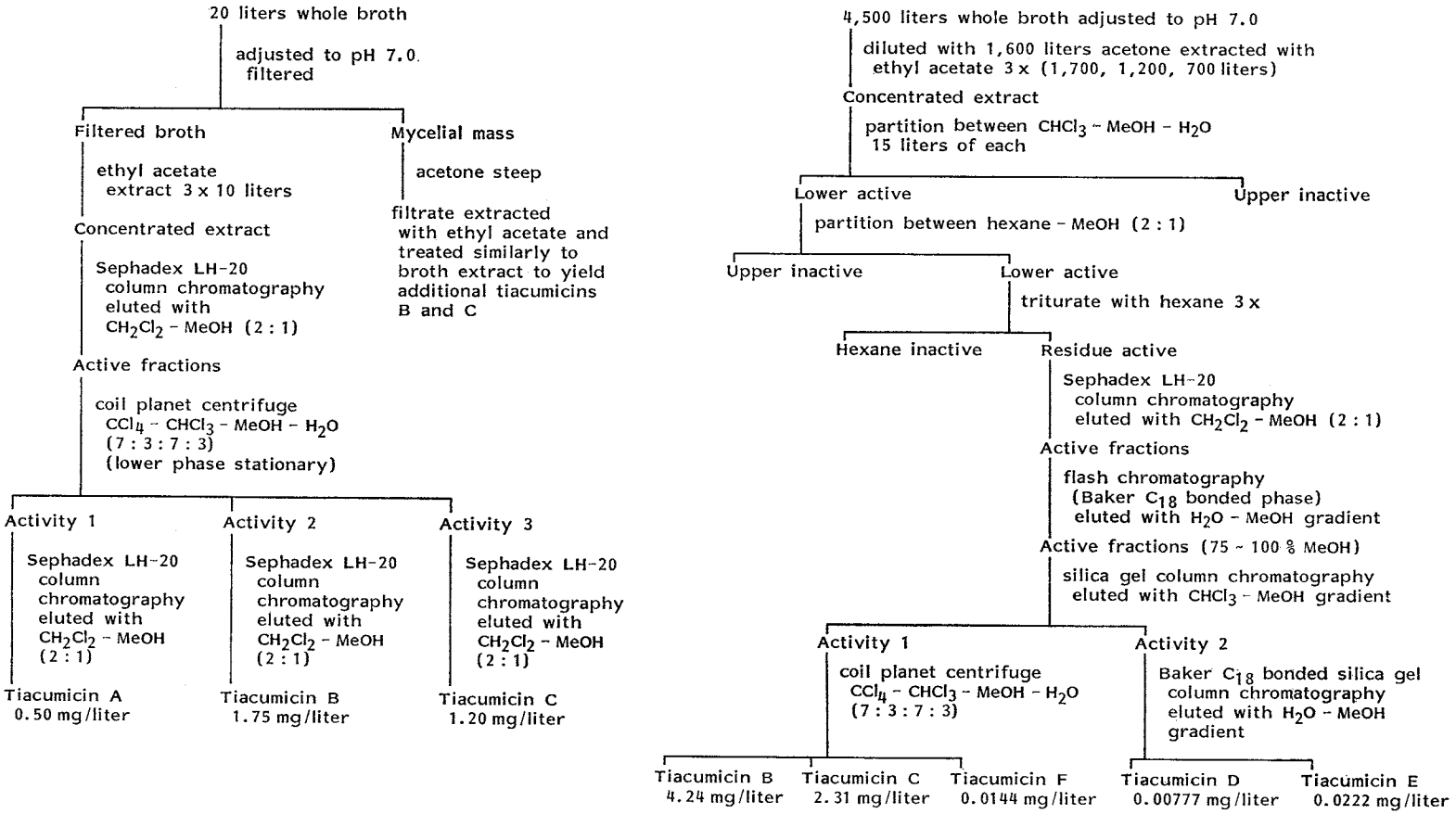
Isolation of Tiacumicins A, B and C

As outlined in Fig. 1, whole broth (20 liters) was adjusted to pH 7 with dilute HCl, then centrifuged and filtered through filter paper to remove the mycelial cake. The filtered broth was extracted three times with ethyl acetate (3×10 liters). Ethyl acetate extracts were combined, dried over sodium sulfate, and concentrated under vacuum to an oily residue. The residue was chromatographed on a Sephadex LH-20 column (1 liter) eluted with CH_2Cl_2 - MeOH (2:1). Active fractions were pooled and subjected to counter-current chromatography of an Ito multilayer coil planet centrifuge employing a CCl_4 - $CHCl_3$ - MeOH - H_2O (7:3:7:3) solvent system (lower phase stationary). Three active bands from the coil planet centrifuge were separately chromatographed on Sephadex LH-20 eluted with CH_2Cl_2 - MeOH (2:1) to yield three pure components; tiacumicins A (10 mg), B (35 mg) and C (24 mg). The mycelial mass was soaked twice in acetone (2×1 liter) which was then filtered off and evaporated to an aqueous concentrate. This aqueous suspension was diluted with distilled water to a volume of 2 liters and extracted three times with ethyl acetate (3×1 liter). Ethyl acetate extracts were combined, dried over sodium sulfate and concentrated to an oily residue. The residue was triturated twice with hexane (2×1 liter) and the hexane layers were discarded leaving an oily residue. Chromatographic processing analogous to that of the broth extract yielded additional tiacumicins B (12 mg) and C (8 mg).

Isolation of Tiacumicins D, E and F

As outlined in Fig. 1, whole broth (4,500 liters) was adjusted to pH 7 with H_2SO_4 and a portion

Fig. 1. Isolation of the tiacumicins.



of the insoluble material removed by centrifugation. The remaining broth and mycelial mass, diluted with acetone (1,600 liters) to lyse the mycelium, was extracted three times with ethyl acetate (1,700, 1,200 and 700 liters). Extracts were combined and concentrated under reduced pressure to an oily residue. The residue was partitioned between $CHCl_3$ - MeOH - H_2O (15 liters of each) and the upper layer discarded. The lower layer was concentrated to an oil and the $CHCl_3$ - MeOH - H_2O partitioning repeated twice. The final lower layer residue was partitioned between MeOH and hexane (6 liters of each). The upper layer was discarded and the lower layer concentrated to a residue. This residue was triturated with hexane four times (4×6 liters) and the hexane was discarded to leave a solid (200 g). A portion (40 g) was chromatographed over a Sephadex LH-20 column (7.5×90 cm) eluted with CH_2Cl_2 - MeOH (2:1). Active fractions were combined and subjected to flash chromatography in a Baker C_{18} column eluted with a step gradient ranging from H_2O to MeOH in 25% increments. Active fractions (75% and 100% MeOH eluates) were combined and concentrated by evaporation under reduced pressure followed by lyophilization to yield a solid residue (10 g). This was chromatographed on a silica gel column (7.5×100 cm) eluted with a step gradient of $CHCl_3$ to $CHCl_3$ - 50% MeOH. Active fractions were combined into two pools each of which was concentrated to a residue (12.5 g and 0.2 g, respectively). The former residue was chromatographed on an Ito multilayer coil planet centrifuge employing a CCl_4 - $CHCl_3$ - MeOH - H_2O (7:3:7:3) solvent system (lower phase stationary) in 10 batches to yield pure tiacumicins B (3.82 g), C (2.08 g) and F (13 mg). The latter residue was chromatographed on Baker C_{18} bonded phase silica gel to yield pure tiacumicins D (7 mg) and E (20 mg).

Structure Determination of Tiacumicin B

The structure of tiacumicin B, the major antibiotic component produced by this culture will be discussed first. For ease of data presentation, the full structure of tiacumicin B (**2**) is given at the onset of this discussion.

Fast atom bombardment mass spectrometry (FAB-MS) in both the negative and positive ion modes established the molecular weight of the lowest abundant isotope of tiacumicin B as *m/z* 1,056. The isotopic distribution pattern of the molecular ion cluster indicated the presence of two chlorine atoms. Proton decoupled and distortionless enhancement by polarization transfer (DEPT) ^{13}C NMR spectra indicated the presence of 52 carbon atoms with 67 attached protons (see Table 1). Although no mass match was possible on the parent ion cluster, mass matching of several fragments suggested a molecular formula of $C_{52}H_{74}O_{18}Cl_2$ indicating in tiacumicin B 15 units of unsaturation and seven exchangeable protons.

A chemical shift correlation map (CSCM) helped to identify many functional groups within tiacumicin B (see Fig. 2 and compiled data in Table 1). In particular, two protons at δ 5.08

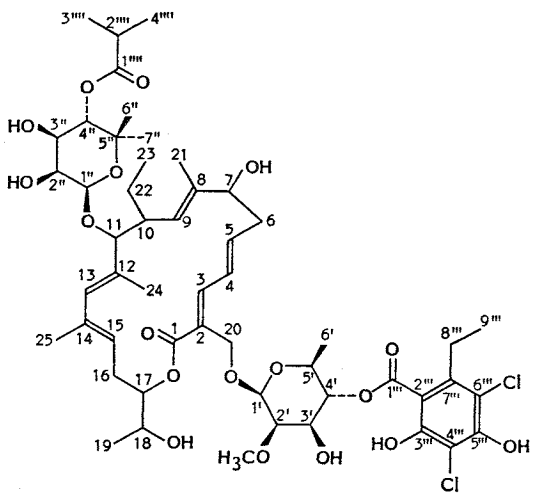


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Table 1. Proton and carbon NMR assignments (in CD_3OD) for tiacumicin B.

Carbon number	Carbon chemical shift (δ)	Proton(s) chemical shift (δ)
1	169.1 (Q)	—
2	125.6 (Q)	—
3	146.3 (CH)	7.21 (d, J=11.4 Hz)
4	128.5 (CH)	6.58 (dd, J=14.5, 11.4 Hz)
5	143.7 (CH)	5.94 (ddd, J=14.5, 9.3, 4.9 Hz)
6	37.3 (CH_2)	2.7 (m), 2.48 (ddd, J=9.3, 7.2, 4.5 Hz)
7	72.8 (CH)	4.21 (m)
8	137.0 (Q)	—
9	124.6 (CH)	5.13 (br d, J=10.0 Hz)
10	42.5 (CH)	2.7 (m)
11	94.3 (CH)	3.69 (d, J=9.7 Hz)
12	136.9 (Q)	—
13	134.6 (CH)	5.82 (br s)
14	136.3 (Q)	—
15	126.8 (CH)	5.56 (br t, J=7.9 Hz)
16	28.4 (CH_2)	2.7 (m), 2.42 (ddd, J=13.9, 7.9, 4.5 Hz)
17	78.6 (CH)	4.72 (ddd, J=6.4, 4.8, 4.5 Hz)
18	68.3 (CH)	4.01 (pentet, J=6.4 Hz)
19	14.6 (CH_3)	1.15 (d, J=6.8 Hz)
20	63.9 (CH_2)	4.42 (d, J=11.5 Hz), 4.60 (d, J=11.5 Hz)
21	15.4 (CH_3)	1.64 (br s)
22	26.9 (CH_2)	1.25 (m), 2.00 (m)
23	11.3 (CH_3)	0.87 (t, J=7.4 Hz)
24	13.9 (CH_3)	1.80 (br s)
25	17.5 (CH_3)	1.75 (br s)
1′	102.2 (CH)	4.62 (br s)
2′	71.6 (CH)	3.52 (br d, J=3.2 Hz)
2′-OCH_3	62.2 (CH_3)	3.55 (s)
3′	73.2 (CH)	3.73 (dd, J=9.8, 3.2 Hz)
4′	76.8 (CH)	5.08 (t, J=9.8 Hz)
5′	82.5 (CH)	3.54 (dq, J=9.8, 6.2 Hz)
6′	18.1 (CH_3)	1.33 (d, J=6.2 Hz)
1″	97.1 (CH)	4.70 (br s)
2″	73.5 (CH)	3.92 (br d, J=3.4 Hz)
3″	70.5 (CH)	3.71 (dd, J=10.2, 3.4 Hz)
4″	75.9 (CH)	5.01 (d, J=10.2 Hz)
5″	74.5 (Q)	—
6″	28.7 (CH_3)	1.14 (s)
7″	18.2 (CH_3)	1.11 (s)
1‴	170.1 (Q)	—
2‴	110.7 (Q)	—
3‴	155.4 (Q)*	—
4‴	108.9 (Q)	—
5‴	155.8 (Q)*	—
6‴	115.7 (Q)	—
7‴	141.9 (Q)	—
8‴	26.5 (CH_2)	2.95 (m)
9‴	20.2 (CH_3)	1.19 (t, J=7.3 Hz)
1⁗	178.4 (Q)	—
2⁗	35.4 (CH)	2.60 (heptet, J=7.0 Hz)
3⁗	19.1 (CH_3)**	1.17 (d, J=7 Hz)
4⁗	19.5 (CH_3)**	1.18 (d, J=7 Hz)

* and ** represent unresolved carbons.
Q: Quaternary.

and 5.01 correlating to carbon atoms at δ 76.8 and 75.9 respectively were assigned as attached to the alkyl carbons of an ester and easily distinguished from an olefinic proton at δ 5.13 which correlated to a carbon atom at δ 124.6. CSCM data also proved valuable in relating carbon atoms through proton-proton connectivity data.

A correlation spectroscopy (COSY) spectrum yielded proton-proton connectivity information which, through the delayed COSY sequence (see Fig. 3), was extended to long range couplings and defined several large portions of the tiacumicin B macrolide ring. These included an $\alpha\beta,\gamma\delta$-unsaturated ester substituted on the α and δ positions (**2a**), a 1,1,3,4-tetrasubstituted diene (**2b**) and a 1,1,2-trisubstituted olefin (**2c**).

Fig. 2. Proton-carbon chemical shift correlation map (CSCM) of tiacumicin B in CD_3OD.

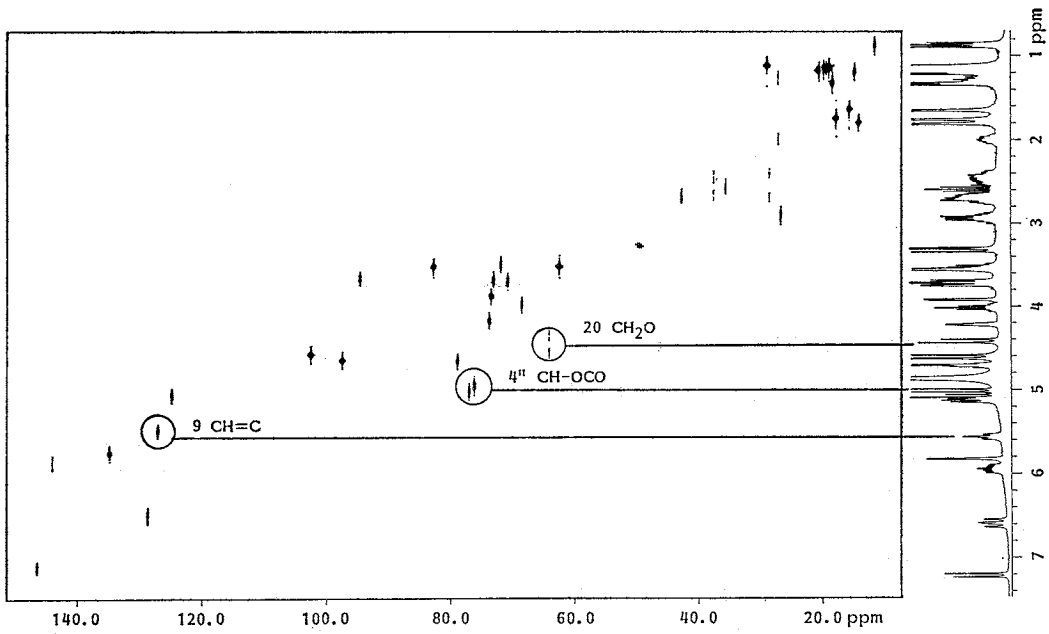
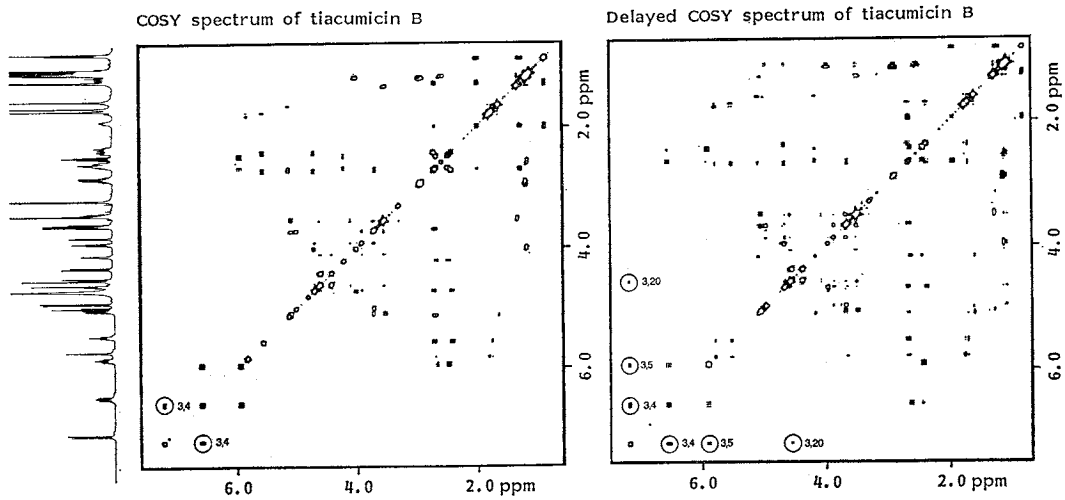
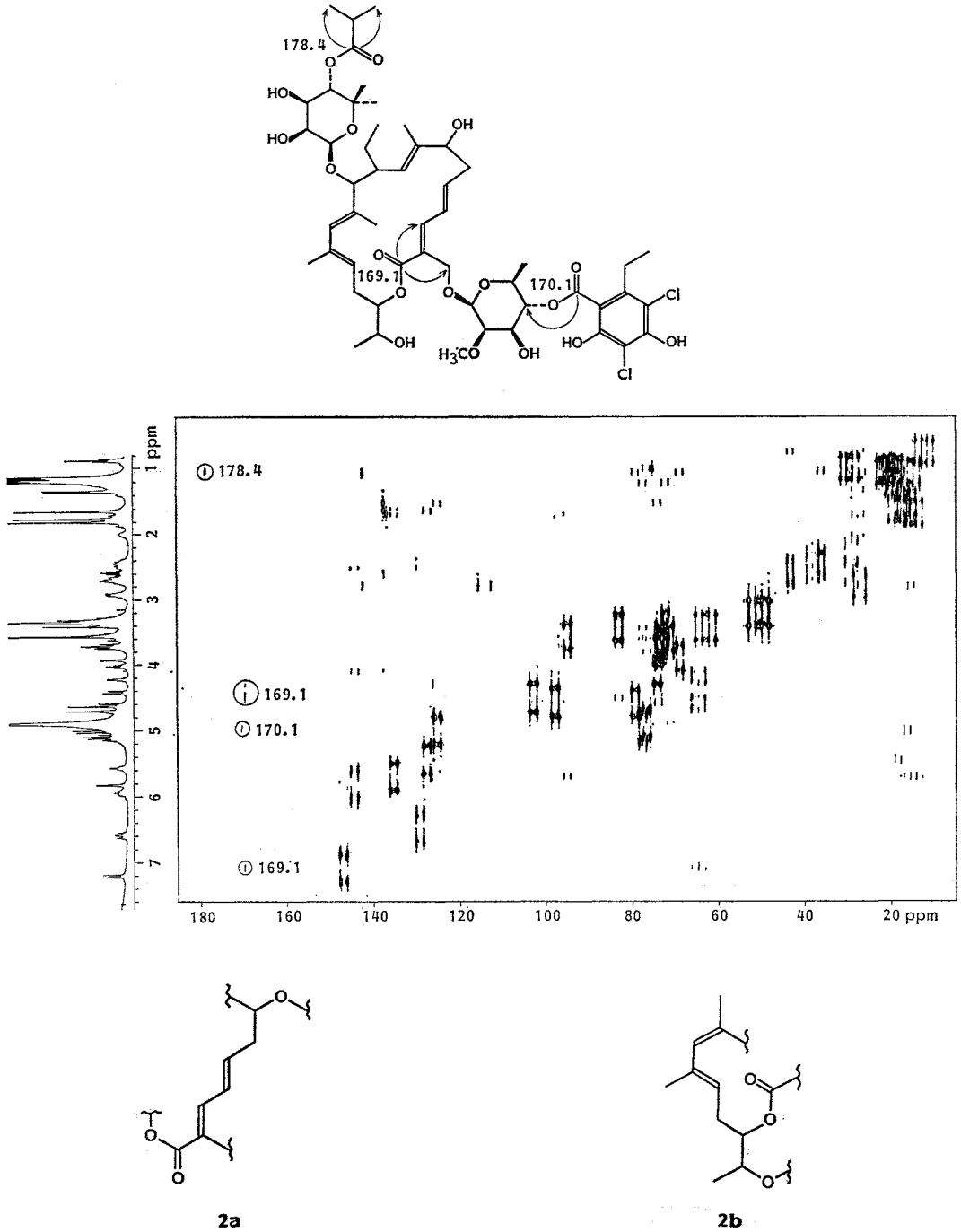


Fig. 3. COSY and DELCOSY spectra of tiacumicin B in CD_3OD.



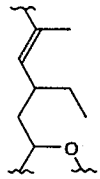
A ^{13}C NMR spectrum of tiacumicin B contained signals attributable to three ester carbonyl carbons at δ 178.4, 170.1 and 169.1. Intense bands in the IR spectrum at 1735, 1695 and 1665 cm^{-1} supported the presence of three ester functionalities associated with varying degrees of unsaturation. A heteronuclear COSY experiment (see Fig. 4) was employed to define coupling between these ester

Fig. 4. Heteronuclear COSY spectrum of tiacumicin B in CD_3OD.

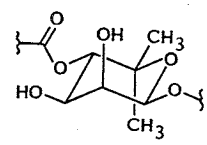


2a

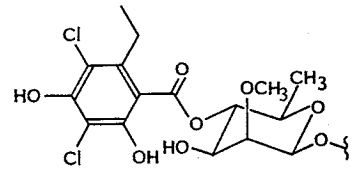
2b



2c



2d



2e

carbonyls and protonated portions of the structure. The carbon signal at δ 178.4 was coupled to the protons of two methyl groups defined by decoupling experiments as part of an isolated isopropyl group. A positive ion mode FAB-MS fragment of *m/z* 231 and a negative ion mode fragment at *m/z* 247 together with COSY information suggested an isobutyrate of a 7 carbon sugar (**2d**).

Mass spectral fragments of *m/z* 409 in the negative ion mode and *m/z* 393 and *m/z* 249 in the positive ion mode FAB-MS were mass matched to $C_{16}H_{19}O_8Cl_2$, $C_{16}H_{19}O_7Cl_2$ and $C_9H_7O_4Cl_2$ respectively. These fragments were attributed to a 6-deoxy hexose esterified with a dichloro substituted aromatic acid isomeric with everninic acid (**2e**). The ester linkage is further supported and the aromatic acid implicated as one having a phenolic hydroxyl group *ortho* to the carbonyl by the 1665 cm^{-1} absorption in the IR spectrum. In addition, a coupling of the proton at position 4 of the 6-deoxy sugar was observed to the carbonyl carbon of δ 170.1 in the heteronuclear COSY spectrum.

Quaternary carbon atoms in the ^{13}C NMR spectrum of tiacumicin B at δ 110.7, 156.4, 108.9, 155.8, 115.7 and 141.9 were in good agreement with calculated values[2] of δ 112.8, 156.4, 107.9, 162.1, 115.7 and 146.5 for carbons 2‴ through 7‴.

Finally, the heteronuclear COSY spectrum showed a connectivity for the remaining carbonyl carbon signal at δ 7.21 previously defined as part of the $\alpha\beta$, $\gamma\delta$-unsaturated ester system (**2c**). This same carbon was also coupled to an isolated CH_2O group. Further, heteronuclear couplings from fully substituted olefinic carbon atoms and supported by nuclear Overhauser experiments (to be discussed later) allowed the 3 major fragments of the aglycone to be connected.

The positions of the free hydroxyl groups and, by difference the site of glycosidic attachment of the sugars was determined by taking advantage of the fact that a carbon atom attached to a hydroxyl group will resonate at a slightly different chemical shift when the proton is replaced by a deuterium. ^{13}C NMR spectra of tiacumicin B were therefore acquired in CD_3OD and CH_3OH separately. By observing which signals shift slightly between the two spectra the carbons bearing hydroxyl groups were distinguished from ether carbons. In this experiment, carbon signals of δ 68.3 (C18) and 72.8 (C7) on the macrolide ring were established as hydroxyl bearing carbons and thus carbons 11 and 20 must be the sites of attachment of the two sugars. Through similar arguments, the methoxyl group whose presence was deduced from signals at δ 62.2 and 3.55 in the ^{13}C NMR and ^1H NMR spectra respectively was placed at position 2 of the 6-deoxy sugar.

Nuclear Overhauser effect (NOE) experiments showed dipolar coupling between the anomeric proton at δ 4.70 (carbon 1″) and a proton at δ 3.69 (carbon 11) placing the 7 carbon sugar at carbon

11 of the macrolide and, by default, the 6-deoxy sugar at carbon 20. In addition, NOE data also established the stereochemistry about the olefinic systems of the macrolide ring. Dipolar couplings were observed between the olefinic protons on carbons 3 and 5, and between the proton on carbon 4 and those on 20 establishing a *trans-trans* diene stereochemistry for this unsaturated ester. Similarly, strong NOE's were observed between protons on carbon atoms 13 and 25, and 24 and 15 establishing an singlet-*cis* configuration for this diene. The absence of an NOE between protons on carbons 9 and 21 although negative evidence suggests that the stereochemistry about this olefin is *trans*.

Structure Determination of Tiacumicins A, C, D, E and F

Tiacumicins C (**3**) and F (**6**) each had the same molecular weight as tiacumicin B. Analysis of the homonuclear and heteronuclear carbon and proton data (see Tables 2 and 3) in conjunction with mass spectral data indicated that these compounds differed from tiacumicin B only in the position of butyrate esterification on the 7 carbon sugar. These changes are best demonstrated in the ^{1}H NMR spectra. The chemical shifts of protons on carbon atoms 2″, 3″ and 4″ in the spectrum of tiacumicin B were changed from δ 3.92, 3.71, and 5.01 to 5.34, 3.72, and 3.44 respectively for tiacumicin C and to 4.01, 4.77, and 3.74 respectively for tiacumicin F with only minor changes in the values of their coupling constants.

Tiacumicin D (**4**), also isomeric with tiacumicin B, differed from B by the position of esterification on the 6-deoxy sugar. Protons on carbon atoms 3′ and 4′ in tiacumicin B had chemical shifts of δ 3.73 and 5.08. These occurred at 4.99 and 3.60 respectively in tiacumicin D. Thus the everninic acid isomer on carbon 4′ in tiacumicin B is attached to carbon 3′ in tiacumicin D.

Tiacumicin E (**5**) had a molecular weight of *m/z* 1,042, or 14 mass units lower than that of tiacumicin B. NMR spectra revealed that the structure of tiacumicin E was identical to that of tiacumicin C except for the ester moiety attached to carbon 2″. The isobutyrate in tiacumicin C with proton signals at δ 2.65 (heptet, 1H, J=7.0 Hz) and 1.18 (d, 3H, J=7.0 Hz) has been replaced in tiacumicin E by a propionate ester moiety as evidenced by two signals at δ 2.46 (q, 2H, J=7.6 Hz) and 1.16 (t, 3H, J=7.6 Hz).

The molecular formula of tiacumicin A (**1**) was established as $C_{34}H_{52}O_9$ by mass matching of the electron impact mass spectral ion at *m/z* 604.3611 (calcd 604.3597). All signals for the aromatic ester and 6-deoxy sugar were absent from the ^{13}C NMR and ^{1}H NMR spectra (see Tables 2 and 3). The esterification pattern in the 7 carbon sugar was the same as that for tiacumicin B, but the ester is an acetate in tiacumicin A. Minor structural changes were also observed in the macrolide ring. Two sites of oxygenation in tiacumicin B appear to be reduced in the tiacumicin A aglycone. Carbon atoms 18 and 20 whose signals appear at δ 68.3 (CH) and 63.9 (CH_2) in tiacumicin B are assigned to signals at δ 26.1 (CH_2) and 11.7 (CH_3) in tiacumicin A. Associated changes in the proton signal assignments (Table 1) are consistent with the formula for tiacumicin A.

Experimental

General Procedures

Optical rotations were measured in 1 dm tubes on a Perkin Elmer Model 241 polarimeter. Melting points were recorded on a Hoover Unimelt and are uncorrected. IR spectra were recorded on a Perkin Elmer 683 dual beam dispersive instrument and UV spectra on a Perkin Elmer Lambda 3B ultraviolet-visible spectrophotometer. NMR spectra were measured on either a General Electric GN300 or GN500 spectrometer with 5 mm probes. The chemical shift correlation maps (CSCM) were

Table 2. ^{13}C NMR assignments for tiacumicins A through F. (Tiacumicin A data recorded in $CDCl_3$, tiacumicins B~F in CD_3OD.)

Carbon number	Tiacumicin A	Tiacumicin B	Tiacumicin C	Tiacumicin D	Tiacumicin E	Tiacumicin F
1	171.3 (Q)	169.1 (Q)	169.1 (Q)	170.0 (Q)	170.4 (Q)	170.1 (Q)
2	124.8 (Q)	125.6 (Q)	125.6 (Q)	126.4 (Q)	126.5 (Q)	126.5 (Q)
3	139.7 (CH)	146.3 (CH)	146.1 (CH)	147.2 (CH)	147.0 (CH)	147.1 (CH)
4	128.3 (CH)	128.5 (CH)	128.5 (CH)	129.4 (CH)	129.4 (CH)	129.4 (CH)
5	137.4 (CH)	143.7 (CH)	143.6 (CH)	144.6 (CH)	144.5 (CH)	144.6 (CH)
6	35.9 (CH_2)	37.3 (CH_3)	37.3 (CH_2)	38.2 (CH_2)	38.2 (CH_2)	38.3 (CH_2)
7	72.1 (CH)	72.8 (CH)	72.8 (CH)	74.4 (CH)	74.4 (CH)	74.4 (CH)
8	135.8 (Q)*	137.0 (Q)	136.9 (Q)	137.9 (Q)	137.9 (Q)	137.9 (Q)
9	122.6 (CH)	124.6 (CH)	124.6 (CH)	125.5 (CH)	125.4 (CH)	125.5 (CH)
10	40.9 (CH)	42.5 (CH)	42.6 (CH)	43.6 (CH)	43.4 (CH)	43.4 (CH)
11	92.6 (CH)	94.3 (CH)	93.1 (CH)	94.1 (CH)	94.0 (CH)	95.0 (CH)
12	135.2 (Q)*	136.9 (Q)	136.8 (Q)	137.8 (Q)	137.7 (Q)	137.8 (Q)
13	133.6 (CH)	134.6 (CH)	135.2 (CH)	136.2 (CH)	136.4 (CH)	137.5 (CH)
14	134.3 (Q)*	136.3 (Q)	135.7 (Q)	136.6 (Q)	136.3 (Q)	137.0 (Q)
15	125.1 (CH)	126.8 (CH)	127.1 (CH)	128.0 (CH)	128.1 (CH)	127.8 (CH)
16	31.0 (CH_2)	28.4 (CH_2)	28.4 (CH_2)	29.2 (CH_2)	29.2 (CH_2)	29.2 (CH_2)
17	74.8 (CH)	78.6 (CH)	78.6 (CH)	79.5 (CH)	79.5 (CH)	79.6 (CH)
18	26.1 (CH_2)	68.3 (CH)	68.4 (CH)	69.2 (CH)	69.2 (CH)	69.2 (CH)
19	9.6 (CH_3)	14.6 (CH_3)	14.5 (CH_3)	15.3 (CH_3)	15.5 (CH_3)	15.5 (CH_3)
20	11.7 (CH_3)	63.9 (CH_2)	63.9 (CH_2)	64.6 (CH_2)	64.8 (CH_2)	64.8 (CH_2)
21	14.6 (CH_3)	15.4 (CH_3)	15.4 (CH_3)	16.3 (CH_3)	16.3 (CH_3)	16.3 (CH_3)
22	25.6 (CH_2)	26.9 (CH_2)	26.6 (CH_2)	27.5 (CH_2)	27.4 (CH_2)	27.7 (CH_2)
23	10.4 (CH_3)	11.3 (CH_3)	11.4 (CH_3)	12.3 (CH_3)	12.2 (CH_3)	12.2 (CH_3)
24	12.9 (CH_3)	13.9 (CH_3)	13.8 (CH_3)	14.7 (CH_3)	14.6 (CH_3)	14.8 (CH_3)
25	16.7 (CH_3)	17.5 (CH_3)	17.5 (CH_3)	18.4 (CH_3)	18.4 (CH_3)	18.4 (CH_3)
1′		102.2 (CH)	102.2 (CH)	102.4 (CH)	103.1 (CH)	103.1 (CH)
2′		71.6 (CH)	71.6 (CH)	81.0 (CH)	72.5 (CH)	72.5 (CH)
2′-OCH_3		62.2 (CH_3)	62.2 (CH_3)	63.1 (CH_3)	63.1 (CH_3)	63.1 (CH_3)
3′		73.2 (CH)	72.8 (CH)	78.9 (CH)	73.1 (CH)	73.7 (CH)
4′		76.8 (CH)	76.9 (CH)	72.2 (CH)	77.6 (CH)	77.7 (CH)
5′		82.5 (CH)	82.4 (CH)	74.9 (CH)	83.3 (CH)	83.4 (CH)
6′		18.1 (CH_3)	18.1 (CH_3)	18.9 (CH_3)	19.0 (CH_3)	19.0 (CH_3)
1″	94.9 (CH)	97.1 (CH)	94.8 (CH)	95.7 (CH)	95.6 (CH)	97.4 (CH)
2″	71.3 (CH)	73.5 (CH)	73.6 (CH)	74.6 (CH)	74.6 (CH)	71.7 (CH)
3″	69.4 (CH)	70.5 (CH)	70.3 (CH)	71.2 (CH)	71.2 (CH)	75.9 (CH)
4″	74.8 (CH)	75.9 (CH)	75.1 (CH)	76.0 (CH)	75.9 (CH)	72.8 (CH)
5″	73.1 (Q)	74.5 (Q)	76.1 (Q)	77.1 (Q)	77.2 (Q)	76.9 (Q)
6″	27.7 (CH_3)	28.7 (CH_3)	28.9 (CH_3)	29.8 (CH_3)	29.7 (CH_3)	29.6 (CH_3)
7″	17.8 (CH_3)	18.2 (CH_3)	17.8 (CH_3)	18.7 (CH_3)	18.7 (CH_3)	18.8 (CH_3)
1‴		170.1 (Q)	170.0 (Q)	171.1 (Q)	170.9 (Q)	171.0 (Q)
2‴		110.7 (Q)	111.5 (Q)	110.2 (Q)	111.8 (Q)	111.9 (Q)
3‴		155.4 (Q)*	155.0 (Q)*	156.1 (Q)*	156.2 (Q)*	156.1 (Q)*
4‴		108.9 (Q)	108.9 (Q)	109.7 (Q)	109.8 (Q)	109.8 (Q)
5‴		155.8 (Q)*	155.2 (Q)*	157.6 (Q)*	156.5 (Q)*	156.3 (Q)*
6‴		115.7 (Q)	115.3 (Q)	116.8 (Q)	116.2 (Q)	116.6 (Q)
7‴		141.9 (Q)	142.0 (Q)	144.1 (Q)	142.8 (Q)	142.8 (Q)
8‴		26.5 (CH_2)	26.4 (CH_2)	27.4 (CH_2)	27.4 (CH_2)	27.4 (CH_2)
9‴		20.2 (CH_3)	20.2 (CH_3)	20.6 (CH_3)	21.1 (CH_3)	21.1 (CH_3)
1⁗	169.1 (Q)	178.4 (Q)	178.6 (Q)	179.6 (Q)	176.9 (Q)	179.8 (Q)
2⁗	20.6 (CH_3)	35.4 (CH)	35.4 (CH)	36.3 (CH)	29.3 (CH_2)	36.2 (CH)
3⁗		19.1 (CH_3)**	19.7 (CH_3)**	20.4 (CH_3)**	10.5 (CH_3)	20.3 (CH_3)**
4⁗		19.5 (CH_3)**	19.5 (CH_3)**	21.1 (CH_3)**		20.4 (CH_3)**

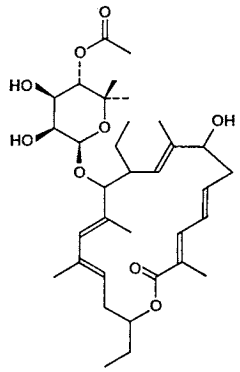
* and ** indicate unresolved carbon assignments.

Q: Quaternary.

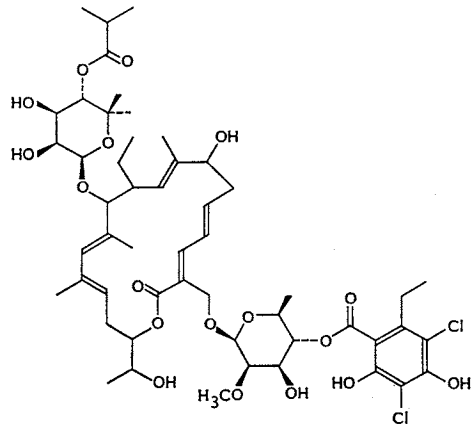
Table 3. ^{1}H NMR assignments for tiacumicins A through F. (Tiacumicin A data recorded in C_6D_6, tiacumicins B~F in CD_3OD, J; Hz.)

Protons on carbon number	Tiacumicin A	Tiacumicin B	Tiacumicin C	Tiacumicin D	Tiacumicin E	Tiacumicin F
3	7.25 (d, 1H, J=11.3)	7.21 (d, 1H, J=11.4)	7.18 (d, 1H, J=11.4)	7.21 (d, 1H, J=11.4)	7.19 (d, 1H, J=11.4)	7.20 (d, 1H, J=11.4)
4	6.25 (ddd, 1H, J= 15.1, 11.3, 1.1)	6.58 (dd, 1H, J= 14.5, 11.4)	6.57 (dd, 1H, J= 14.5, 11.4)	6.57 (dd, 1H, J= 14.5, 11.4)	6.58 (dd, 1H, J= 14.5, 11.4)	6.58 (dd, 1H, J= 14.5, 11.4)
5	5.67 (ddd, 1H, J= 15.1, 10.3, 4.7)	5.94 (ddd, 1H, J= 14.5, 9.3, 4.9)	5.91 (ddd, 1H, J= 14.5, 9.3, 4.9)	5.93 (ddd, 1H, J= 14.5, 9.9, 5.1)	5.92 (ddd, 1H, J= 14.5, 9.7, 5.3)	5.94 (ddd, 1H, J= 14.5, 9.8, 5.2)
6	2.34 (m, 1H)	2.70 (m, 1H)	2.64 (m, 1H)	2.66 (m, 1H)	2.67 (m, 1H)	2.67 (m, 1H)
	2.15 (m, 1H)	2.48 (ddd, 1H, J= 9.3, 7.2, 3.5)	2.47 (m, 1H)	2.46 (m, 1H)	2.45 (m, 1H)	2.48 (m, 1H)
7	3.97 (m, 1H)	4.21 (m, 1H)	4.19 (m, 1H)	4.21 (m, 1H)	4.20 (m, 1H)	4.21 (m, 1H)
9	5.21 (br d, 1H, J=10.3)	5.13 (br d, 1H, J=10.0)	5.10 (br d, 1H, J=10.0)	5.10 (br d, 1H, J=9.8)	5.10 (br d, 1H, J=10.6)	5.12 (br d, 1H, J=10.6)
10	2.66 (ddd, 1H, J= 10.3, 9.7, 5.7)	2.70 (m, 1H)	2.54 (m, 1H)	2.54 (qd, 1H, J=9.8, 2.1)	2.57 (m, 1H)	2.69 (m, 1H)
11	3.45 (d, 1H, J=9.7)	3.69 (d, 1H, J=9.7)	3.64 (d, 1H, J=9.7)	3.66 (d, 1H, J=9.8)	3.67 (d, 1H, J=9.8)	3.69 (d, 1H, J=9.8)
13	5.58 (br s, 1H)	5.82 (br s, 1H)	5.83 (br s, 1H)	5.85 (br s, 1H)	5.85 (br s, 1H)	5.85 (br s, 1H)
15	5.53 (br t, 1H, J=8.2)	5.56 (br t, 1H, J=7.9)	5.56 (br t, 1H, J=7.9)	5.58 (br t, 1H, J=8.1)	5.58 (br t, 1H, J=8.0)	5.58 (br t, 1H, J=8.1)
16	2.48 (ddd, 1H, J= 11.1, 8.2, 4.5)	2.70 (m, 1H)	2.72 (ddd, 1H, J= 12.2, 7.9, 4.8)	2.74 (m, 1H)	2.73 (m, 1H)	2.72 (m, 1H)
	2.18 (m, 1H)	2.42 (ddd, 1H, J= 13.9, 7.9, 4.5)	2.43 (ddd, 1H, J= 12.2, 7.9, 4.4)	2.44 (m, 1H)	2.45 (m, 1H)	2.43 (m, 1H)
17	4.99 (m, 1H)	4.72 (ddd, 1H, J= 6.4, 4.8, 4.5)	4.71 (ddd, 1H, J= 6.4, 4.8, 4.4)	4.21 (m, 1H)	4.72 (m, 1H)	4.72 (m, 1H)
18	1.85 (m, 1H)	4.01 (pentet, 1H, J=6.4)	4.03 (pentet, 1H, J=6.4)	4.03 (pentet, 1H, J=6.4)	4.03 (pentet, 1H, J=6.3)	4.02 (m, 1H)
	1.58 (m, 1H)					
19	0.82 (t, 3H, J=7.4)	1.15 (d, 1H, J=6.4)	1.18 (d, 3H, J=6.4)	1.18 (d, 3H, J=6.4)	1.17 (d, 3H, J=7.4)	1.17 (d, 3H, J=7.0)
20	1.95 (br s, 3H)	4.62 (d, 1H, J=11.5)	4.59 (d, 1H, J=11.5)	4.59 (d, 1H, J=11.5)	4.60 (d, 1H, J=11.5)	4.61 (d, 1H, J=11.5)
		4.42 (d, 1H, J=11.5)	4.42 (d, 1H, J=11.5)	4.43 (d, 1H, J=11.5)	4.42 (d, 1H, J=11.5)	4.42 (d, 1H, J=11.5)

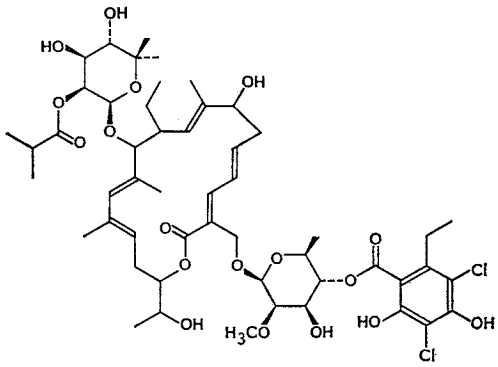
21	1.36 (br s, 3H)	1.64 (br s, 3H)	1.61 (br s, 3H)	1.62 (br s, 3H)	1.63 (br s, 3H)	1.64 (br s, 3H)
22	1.85 (m, 1H)	2.00 (m, 1H)	1.84 (m, 1H)	1.85 (m, 1H)	1.82 (m, 1H)	1.97 (m, 1H)
	1.30 (m, 1H)	1.25 (m, 1H)	1.18 (m, 1H)	1.14 (m, 1H)	1.12 (m, 1H)	1.24 (m, 1H)
23	0.95 (t, 3H, J=7.4)	0.87 (t, 3H, J=7.4)	0.80 (t, 3H, J=7.4)	0.80 (t, 3H, J=7.4)	0.81 (t, 3H, J=7.4)	0.85 (t, 3H, J=7.4)
24	1.90 (br s, 3H)	1.80 (br s, 3H)	1.76 (br s, 3H)	1.77 (br s, 3H)	1.77 (br s, 3H)	1.80 (br s, 3H)
25	1.70 (br s, 3H)	1.75 (br s, 3H)	1.76 (br s, 3H)	1.77 (br s, 3H)	1.76 (br s, 3H)	1.77 (br s, 3H)
1′		4.62 (br s, 1H)	4.63 (br s, 1H)	4.67 (s, 1H)	4.63 (br s, 1H)	4.63 (s, 1H)
2′		3.52 (br d, 1H, J=3.3)	3.54 (br d, 1H, J=3.4)	3.79 (d, 1H, J=3.4)	3.53 (d, 1H, J=3.5)	3.55 (d, 1H, J=3.3)
2′-OCH_3		3.55 (s, 3H)	3.53 (s, 3H)	3.55 (s, 3H)	3.60 (s, 3H)	3.55 (s, 3H)
3′		3.73 (dd, 1H, J=9.9, 3.3)	3.73 (dd, 1H, J=9.8, 3.4)	4.99 (dd, 1H, J=9.8, 3.4)	3.74 (dd, 1H, J=9.7, 3.5)	3.73 (dd, 1H, J=9.8, 3.3)
4′		5.08 (t, 1H, J=9.9)	5.08 (t, 1H, J=9.8)	3.60 (dd, 1H, J=9.8, 9.4)	5.08 (t, 1H, J=9.7)	5.08 (t, 1H, J=9.8)
5′		3.54 (dq, 1H, J=9.9, 6.1)	3.53 (d, 1H, J=9.8, 6.2)	3.36 (dq, 1H, J=9.4, 6.2)	3.52 (dq, 1H, J=9.7, 6.1)	3.53 (dq, 1H, J=9.8, 6.1)
6′		1.33 (d, 3H, J=6.1)	1.33 (d, 3H, J=6.2)	1.36 (d, 3H, J=6.2)	1.33 (d, 3H, J=6.1)	1.33 (d, 3H, J=6.1)
1″	4.26 (d, 1H, J=1.3)	4.70 (br s, 1H)	4.76 (d, 1H, J=1.2)	4.78 (d, 1H, J=1.2)	4.79 (d, 1H, J=1.2)	4.71 (s, 1H)
2″	3.81 (br d, 1H, J=2.9)	3.92 (br d, 1H, J=3.3)	5.34 (dd, 1H, J=3.4, 1.2)	5.36 (dd, 1H, J=3.5, 1.2)	5.37 (dd, 1H, J=3.5, 1.2)	4.01 (br d, 1H, J=3.2)
3″	3.58 (br dd, 1H, J=10.5, 2.9)	3.71 (dd, 1H, J=10.2, 3.3)	3.72 (dd, 1H, J=10.0, 3.4)	3.74 (dd, 1H, J=10.2, 3.5)	3.75 (dd, 1H, J=9.9, 3.5)	4.77 (dd, 1H, J=10.6, 3.2)
4″	5.40 (d, 1H, J=10.5)	5.01 (d, 1H, J=10.2)	3.44 (d, 1H, J=10.0)	3.43 (d, 1H, J=10.2)	3.43 (d, 1H, J=9.9)	3.74 (d, 1H, J=10.6)
6″	1.20 (s, 3H)	1.14 (s, 3H)	1.25 (s, 3H)	1.26 (s, 3H)	1.26 (s, 3H)	1.25 (s, 3H)
7″	1.02 (s, 3H)	1.11 (s, 3H)	1.08 (s, 3H)	1.09 (s, 3H)	1.09 (s, 3H)	1.12 (s, 3H)
8‴		2.95 (m, 2H)	2.93 (m, 2H)	3.05 (q, 2H, J=7.4)	2.94 (m, 2H)	2.93 (m, 2H)
9‴		1.19 (t, 3H, J=7.3)	1.19 (t, 3H, J=7.0)	1.22 (d, 3H, J=7.4)	1.19 (t, 3H, J=7.2)	1.19 (t, 3H, J=7.0)
2⁗	1.75 (s, 3H)	2.60 (heptet, 1H, J=7.0)	2.65 (heptet, 1H, J=7.0)	2.67 (heptet, 1H, J=6.9)	2.46 (q, 2H, J=7.6)	2.65 (pentet, 1H, J=6.9)
3⁗		1.18 (d, 3H, J=7.0)	1.18 (d, 3H, J=7.0)	1.22 (d, 3H, J=6.9)	1.16 (t, 3H, J=7.6)	1.19 (d, 3H, J=6.9)
4⁗		1.17 (d, 3H, J=7.0)	1.18 (d, 3H, J=7.0)	1.21 (d, 3H, J=6.9)		1.18 (d, 3H, J=6.9)



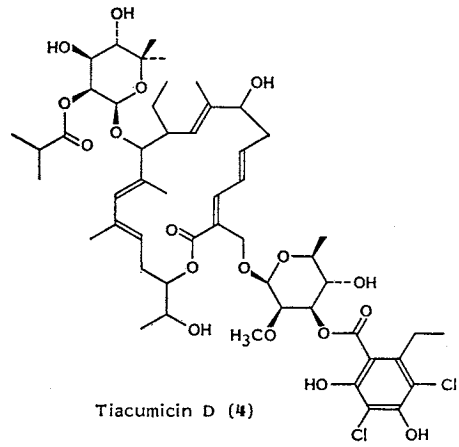
Tiacumicin A (**1**)



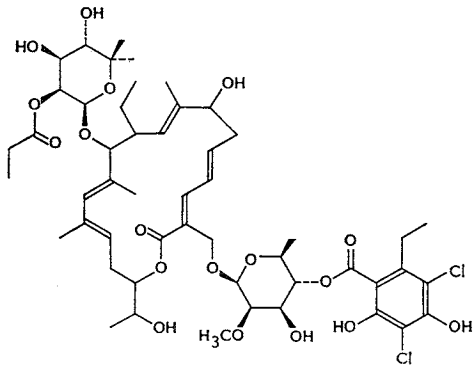
Tiacumicin B (**2**)



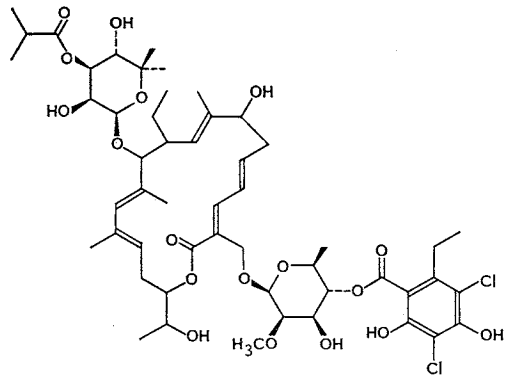
Tiacumicin C (**3**)



Tiacumicin D (**4**)



Tiacumicin E (**5**)



Tiacumicin F (**6**)

acquired by the method of BAX and MORRIS[3] and the DEPT (distortionless enhancement of NMR signals by polarization transfer) by that of DODDRELL *et al.*[4] Delayed correlation spectroscopy studies (DELCOSY) were carried out by the method of BAX and FREEMAN.[5] NOE experiments were acquired by the CAMEL technique of BOTHNER-BY *et al.*[6] Heteronuclear correlation spectroscopy

experiments (FUCOUP) were performed employing a modification of the method of BODENHAUSEN and FREEMAN.[7] Mass spectra were measured on a Kratos MS-50 spectrometer.

Tiacumicin A: $[\alpha]_D^{25}$ +41° (*c* 0.10, MeOH) was a clear oil. A molecular weight of *m/z* 604.3611 was established by electron impact mass spectroscopy. An UV spectrum in MeOH contained bands at λ_{max} nm (ε) 203 (6,785), 235 (6,410), 266 (4,960). These bands were unchanged upon addition of acid or base. An IR spectrum measured neat contained bands at: 3480, 2930, 1735, 1700, 1460, 1370, 1240, 1150, 1115, 1080, 1040 cm^{-1}. See tables for ^1H and ^{13}C NMR data.

Tiacumicin B: $[\alpha]_D^{25}$ −6.5° (*c* 8.2, MeOH) had a melting point of 143~145°C. A molecular weight of *m/z* 1,056 was established by FAB positive and negative ion mass spectrometry. An UV spectrum in MeOH contained bands λ_{max} nm (ε) 206 (13,940), 229 (17,430), 266 (9,300), 314 (2,965), in MeOH - HCl at λ_{max} nm (ε) 206 (14,815), 229 (17,778), 266 (9,630), 314 (870) and in MeOH - NaOH at λ_{max} nm (ε) 204 (32,500), 232 (17,110), 235 (sh), 255 (sh), 270 (sh), 314 (8,220) (see Fig. 4). An IR spectrum measured in KBr contained bands at: 3565, 3502, 2978, 2935, 2880, 1735, 1695, 1665, 1590, 1465, 1455, 1410, 1402, 1380, 1370, 1320, 1310, 1240, 1215, 1195, 1140, 1110, 1065, 1020 cm^{-1}. See tables for ^1H and ^{13}C NMR data.

Tiacumicin C: $[\alpha]_D^{25}$ −8.6° (*c* 15.8, MeOH) had a melting point of 142~143°C. A molecular weight of *m/z* 1,056 was established by FAB positive ion mass spectrometry. An UV spectrum in MeOH contained bands at λ_{max} nm (ε) 206 (25,096), 228 (29,630), 267 (15,410), 315 (6,300), in MeOH - HCl at λ_{max} nm (ε) 206 (25,740), 228 (29,630), 267 (15,555), 315 (1,222), and in MeOH - NaOH at λ_{max} nm (ε) 203 (29,815), 234 (26,500), 240 (sh), 255 (sh), 276 (sh), 315 (11,555). An IR spectrum measured in KBr contained bands at: 3665, 2990, 2945, 2880, 1735, 1695, 1650, 1595, 1470, 1455, 1415, 1405, 1390, 1370, 1310, 1250, 1230, 1200, 1165, 1145, 1115, 1090, 1070, 1025 cm^{-1}. See tables for ^1H and ^{13}C NMR data.

Tiacumicin D: $[\alpha]_D^{25}$ −5.6° (*c* 0.47, MeOH) had a melting point of 141~145°C. A molecular weight of *m/z* 1,056 was established by FAB positive ion mass spectrometry. An UV spectrum in MeOH contained bands at λ_{max} nm (ε) 205 (sh), 229 (15,957), 266 (8,342),3 14 (4,464), in MeOH - HCl at λ_{max} nm (ε) 205 (sh), 229 (17,200), 266 (9,440), 314 (1,050), and in MeOH - NaOH at λ_{max} nm (ε) 203 (sh), 233 (13,878), 242 (sh), 269 (sh), 314 (5,456) (see Fig. 10). An IR spectrum measured in KBr contained bands at: 3436, 2974, 2933, 2874, 1716, 1700, 1640, 1590, 1508, 1456, 1383, 1308, 1251, 1210, 1071 cm^{-1}. See tables for ^1H and ^{13}C NMR data.

Tiacumicin E: $[\alpha]_D^{25}$ −3.2° (*c* 1.3, MeOH) had a melting point of 138~141°C. A molecular weight of *m/z* 1,042 was established by FAB positive ion mass spectrometry. An UV spectrum in MeOH contained bands at λ_{max} nm (ε) 205 (sh), 228 (39,444), 266 (24,853), 315 (10,170), in MeOH - HCl at λ_{max} nm (ε) 205 (40,700), 228 (48,470), 266 (25,730), 315 (1,795), and in MeOH - NaOH at λ_{max} nm (ε) 206 (sh), 234 (43,680), 240 (sh), 255 (sh), 270 (sh), 315 (16,750). An IR spectrum measured in KBr contained bands at: 3444, 2976, 2935, 2876, 1712, 1700, 1640, 1590, 1456, 1380, 1314, 1247, 1209, 1200, 1087, 1068, 1026 cm^{-1}. See tables for ^1H and ^{13}C NMR data.

Tiacumicin F: $[\alpha]_D^{25}$ +5.8° (*c* 0.66, MeOH) had a melting point of 141~143°C. A molecular weight of *m/z* 1,056 was established by FAB positive ion mass spectrometry. An UV spectrum in MeOH contained bands at λ_{max} nm (ε) 204 (16,245), 228 (20,675), 266 (10,127), 315 (4,747), in MeOH - HCl at λ_{max} nm (ε) 204 (17,510), 228 (21,097), 266 (11,392), 315 (1,055) and in MeOH - NaOH at λ_{max} nm (ε) 202 (sh), 234 (19,304), 240 (sh), 255 (sh), 315 (7,226). An IR spectrum measured in $CDCl_3$ contained bands at: 3479, 2975, 2934, 2875, 1709, 1696, 1641, 1588, 1515, 1456, 1381, 1314, 1249, 1200, 1144, 1067, 1022, 908 cm^{-1}. See tables for ^1H and ^{13}C NMR data.

Addendum in Proof

The tiacumicins from *Dactylosporangium aurantiacum* appear to have members in common with antibiotics from two other acinomycetes. The clostomicins from *Micromonospora echinospora* subsp. *armeniaca* were discovered at the Kitasato Institute. Their report,[8] published at the same time as we presented this work at the 26th ICAAC Meeting,[9,10] describes the production, isolation, and spectral characteristics of five members of the clostomicin complex. The published spectra would indicate that clostomicin A is identical to tiacumicin F and clostomicin B_2 to tiacumicin C. Clostomicins C

and D have no known counterpart nor do tiacumicins A, D and E. Clostomicin B_1 and tiacumicin B are identical to the *Actinoplanes deccanensis* produced antibiotic, lipiarmycin, reported earlier by Lepetit scientists.[11~15]

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