STUDIES ON ANTIBIOTIC SF-837, A NEW ANTIBIOTIC. II

CHEMICAL STRUCTURE OF ANTIBIOTIC SF-837

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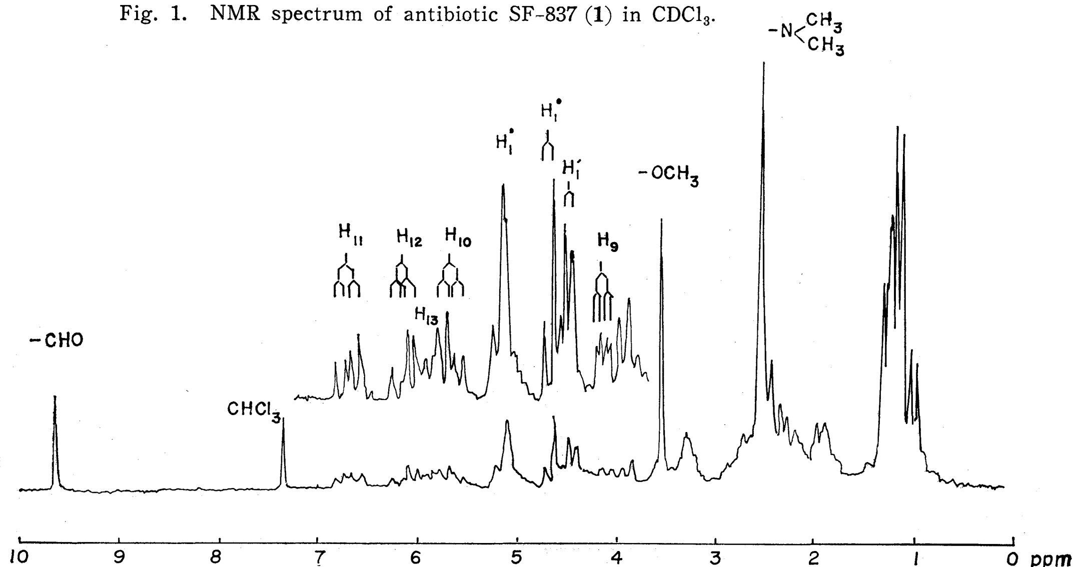
The structure of a new macrolide antibiotic SF-837 was determined as **1** by physico-chemical methods in conjunction with chemical means. Mass spectra of SF-837 and related compounds including leucomycins were accounted for in terms of structures.

Antibiotic SF-837 is a new macrolide having potential utility as a chemotherapeutic agent. Its fermentation, isolation and characterization have been reported in the preceding paper[1]. The structural study of this new antibiotic is the subject of the present paper.

Physico-chemical Studies

The gross structure of antibiotic SF-837 (**1**) was first revealed from the physico-chemical investigations of the intact antibiotic. As described in the preceding paper[1], mass spectrometry and microanalysis of **1** established its molecular formula as $C_{41}H_{67}\cdot NO_{15}$. The IR spectrum disclosed the presence of hydroxy group (3500 cm^{-1}), lactone and/or ester carbonyl group (1735 cm^{-1}), aldehyde C-H bond (2715 cm^{-1}) and C=C double

Fig. 1. NMR spectrum of antibiotic SF-837 (**1**) in $CDCl_3$.



bond (1630 cm^{-1}). A strong UV absorption band at 232 mμ (ε, 26,000) with vibrational fine structure suggested that α, β, γ, δ-unsaturated alcohol might be responsible for the chromophore.

Chart 1. Structure of antibiotic SF-837 (**1**).

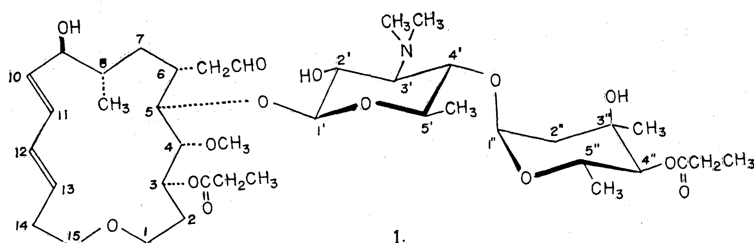
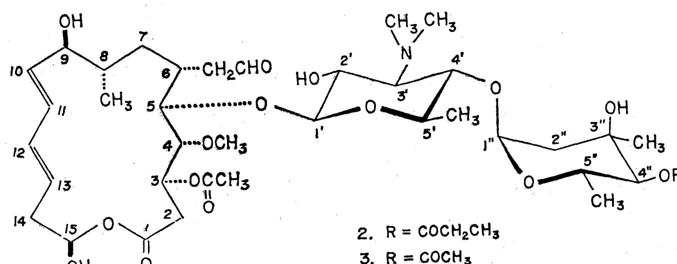
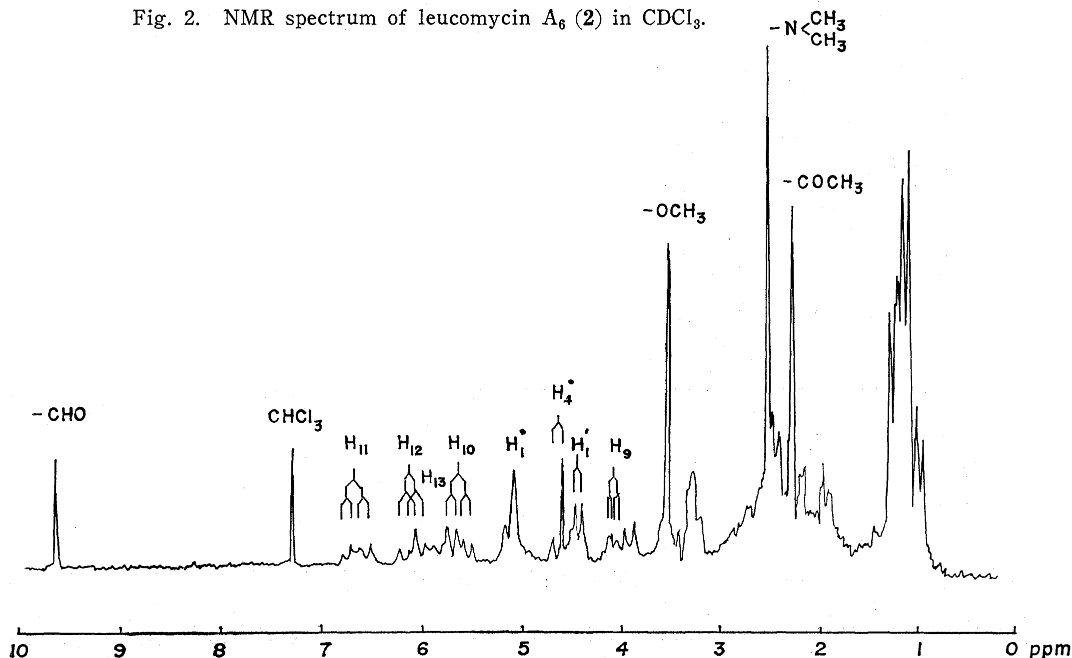


Chart 2. Structure of leucomycin A_6 (**2**) and leucomycin A_8 (**3**).



The NMR spectrum taken in $CDCl_3$ (Fig. 1) displayed in the lowest field a singlet due to an aldehyde proton at δ 9.65, which was changed to a triplet at δ 7.4 in the spectrum of the thiosemicarbazone derivative[1]. Multiplets at δ 5.6~6.8, a singlet at δ 3.53 and a singlet at δ 2.53 were assigned to four olefinic protons, a methoxyl and a dimethylamino groups, respectively. We have noted, at this stage, that the whole feature of the NMR spectrum as well as those of UV and IR spectra were very similar to those reported for leucomycins[2], in particular those bearing an acetyloxy group at C-3 of the sixteen membered macrocyclic lactone. As an example, the NMR spectrum of leucomycin A_6 (**2**) is illustrated in Fig. 2, together with spectral assignment reported by ŌMURA and

Fig. 2. NMR spectrum of leucomycin A_6 (**2**) in $CDCl_3$.



coworkers[3]. Most of the signals of leucomycin A_6 (2) were reproduced as such in the spectrum of SF-837 (1) in Fig. 1, though the latter lacked in an acetyl signal that was presented at δ 2.30 in the leucomycin spectrum. In this connection, it was demonstrated by GLC that SF-837 (1) contained two propionyl groups in its molecule[1], while leucomycin A_6 (2) contained acetyl and propionyl groups. The structural similarity between SF-837 and leucomycins was further substantiated by analogous NMR spectra of the di-O-acetyl derivatives. The newly introduced acetyl signals of di-O-acetyl SF-837 (4)[1] and di-O-acetyl leucomycin A_6 (5) had the same chemical shift at δ 2.04 in $CDCl_3$, suggesting that the new acetyl groups were located on comparable positions in the respective molecules (C-9 and C-2′). The chemical shift of a dimethylamino group of 4 was

Chart 3. Structure of di-O-acetyl SF-837 (4), di-O-acetyl leucomycin A_6 (5) and di-O-acetyl leucomycin A_8 (6).

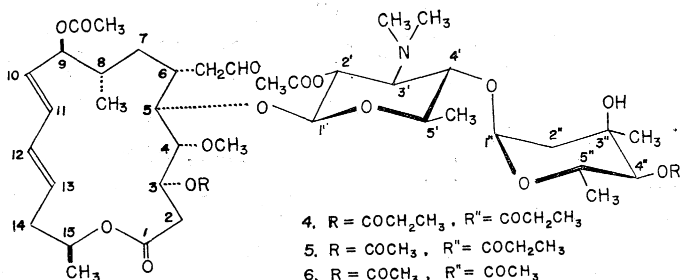


Fig. 3. Mass spectrum of antibiotic SF-837 at 190°C and 210°C.

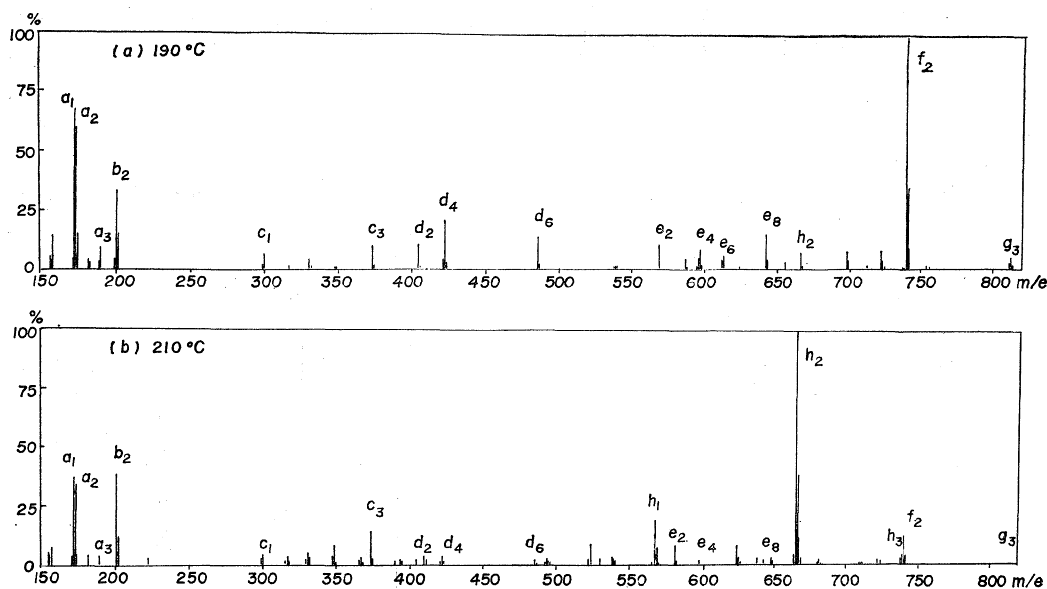


Fig. 4. Mass spectrum of leucomycin A_6 at 180°C.

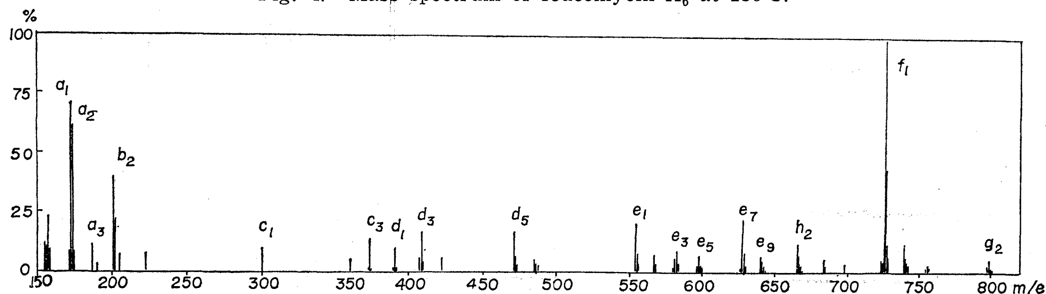


Fig. 5. Mass spectrum of leucomycin A_8 at 190°C.

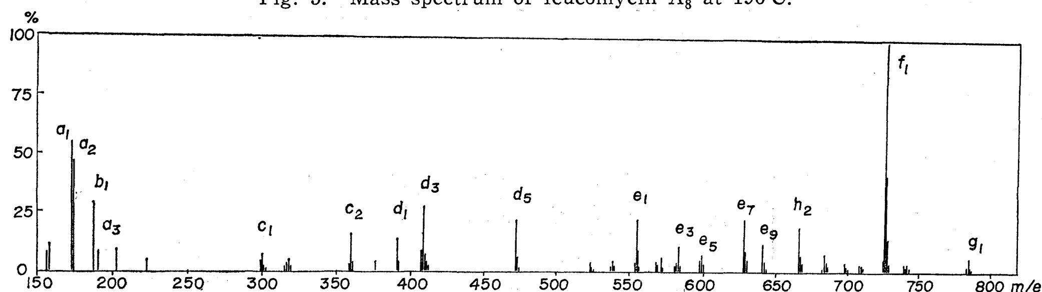


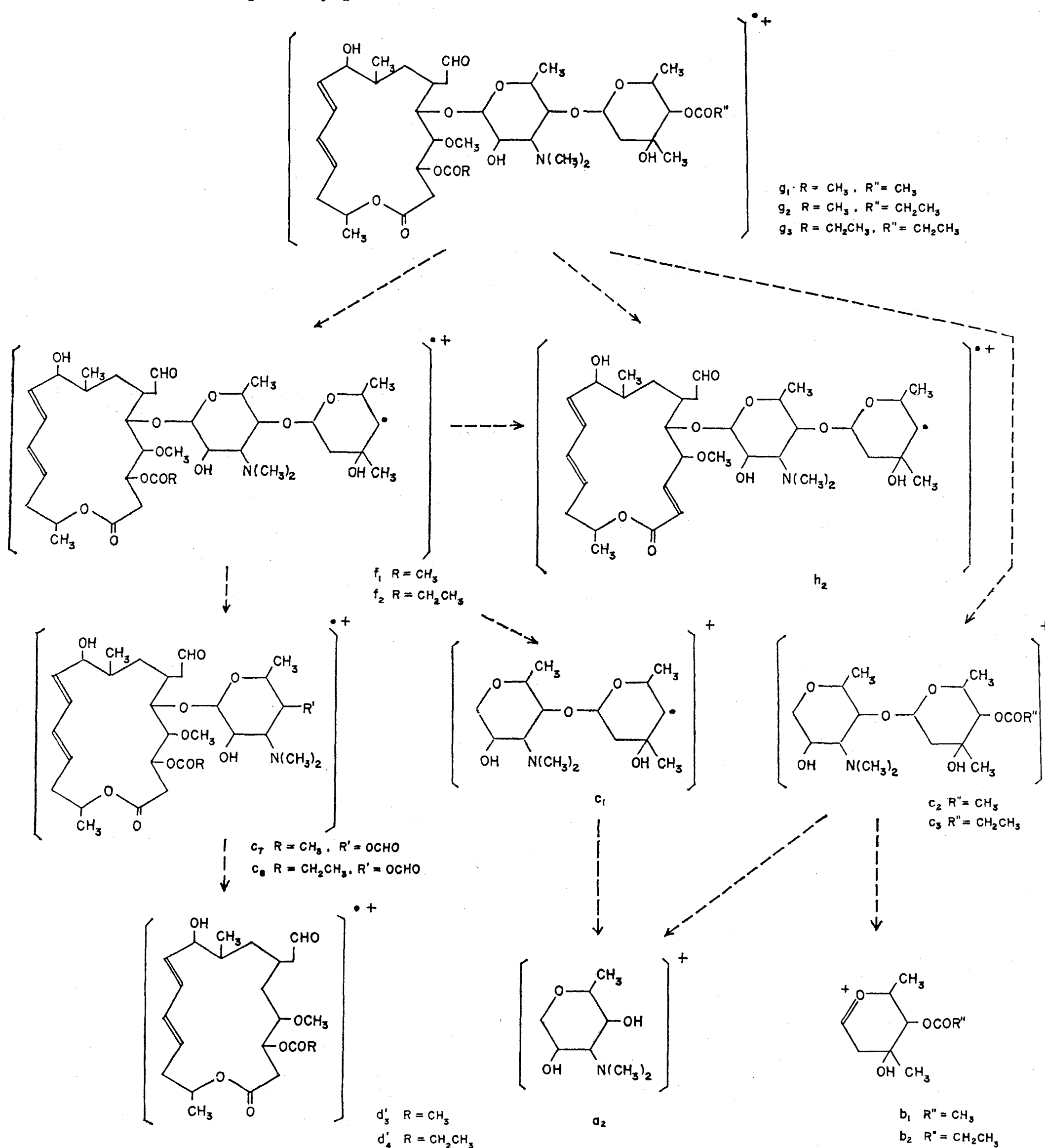
Table 1. Mass fragment ions of antibiotic SF-837 (**1**), leucomycin A_6 (**2**) and A_8 (**3**)

Fragment ion	Nominal Mass (m/e)			Composition	Structure assignment
	1	**2**	**3**		
$\mathbf{a}_1$	173	173	173	$C_8H_{15}NO_3$	Deoxymycaminose
$\mathbf{a}_2$	174	174	174	$C_8H_{16}NO_3$	Deoxymycaminose plus 1
$\mathbf{a}_3$	190	190	190	$C_8H_{16}NO_4$	Mycaminose plus 1
$\mathbf{b}_1$			187	$C_9H_{15}O_4$	Acetyl deoxymycarose
$\mathbf{b}_2$	201	201		$C_{10}H_{17}O_4$	Propionyl-deoxymycarose
$\mathbf{c}_1$	300	300	300	$C_{15}H_{26}NO_5$	Deoxymycarosyl-deoxymycaminose
$\mathbf{c}_2$			360	$C_{17}H_{30}NO_7$	Acetyl-mycarosyl-deoxymycaminose
$\mathbf{c}_3$	374 (376)*	374		$C_{18}H_{32}NO_7$	Propionyl-mycarosyl-deoxymycaminose
$\mathbf{d}_1$		391	391	$C_{22}H_{31}O_6$	Acetyl-deoxylactone minus H_2O
$\mathbf{d}_2$	405			$C_{23}H_{33}O_6$	Propionyl-deoxylactone minus H_2O
$\mathbf{d}_3$		409	409	$C_{22}H_{33}O_7$	Acetyl-deoxylactone
$\mathbf{d}_4$	423 (424)			$C_{23}H_{35}O_7$	Propionyl-deoxylactone
$\mathbf{d}_5$		472	472		Acetyl-deoxylactone plus 63
$\mathbf{d}_6$	486 (488)				Propionyl-deoxylactone plus 63
$\mathbf{e}_1$		555	555		Deoxymycaminosyl-acetyl-lactone minus CO
$\mathbf{e}_2$	569 (571)			$C_{30}H_{51}NO_9$	Deoxymycaminosyl-propionyl-lactone minus CO
$\mathbf{e}_3$		583	583		Deoxymycaminosyl-acetyl-lactone
$\mathbf{e}_4$	597 (599)			$C_{31}H_{51}NO_{10}$	Deoxymycaminosyl-propionyl-lactone
$\mathbf{e}_5$		599	599		Mycaminosyl-acetyl-lactone
$\mathbf{e}_6$	613 (615)			$C_{31}H_{51}NO_{11}$	Mycaminosyl-propionyl-lactone
$\mathbf{e}_7$		628	628		Mycaminosyl-acetyl-lactone plus CHO
$\mathbf{e}_8$	642 (645)			$C_{32}H_{52}NO_{12}$	Mycaminosyl-propionyl-lactone plus CHO
$\mathbf{e}_9$		641	641		Mycaminosyl-acetyl-lactone plus C_2H_2O
$\mathbf{e}_{10}$	655 (657)			$C_{33}H_{53}NO_{12}$	Mycaminosyl-propionyl-lactone plus C_2H_2O
$\mathbf{f}_1$		726	726		Molecular ion minus acetyloxy (or propionyloxy)
$\mathbf{f}_2$	740 (743)			$C_{38}H_{62}NO_{13}$	Molecular ion minus propionyloxy
$\mathbf{g}_1$			785		Molecular ion
$\mathbf{g}_2$		799			Molecular ion
$\mathbf{g}_3$	813 (816)			$C_{41}H_{67}NO_{15}$	Molecular ion
$\mathbf{h}_1$	568	568	568	$C_{30}H_{50}NO_9$	$\mathbf{e}_8$ ($\mathbf{e}_7$) minus propionic acid (acetic acid)
$\mathbf{h}_2$	666 (669)	666	666	$C_{35}H_{56}NO_{11}$	$\mathbf{f}_2$ ($\mathbf{f}_1$) minus propionic acid (acetic acid)
$\mathbf{h}_3$	739				$\mathbf{g}_3$ minus propionic acid

* Numbers in parentheses indicated m/e of the deuterated product.

Chart 4. Structures of principal fragment ions of SF-837 (**1**), leucomycin A_6 (**2**) and A_8 (**3**).*

* Owing to the absence of metastable ions, fragmentation pathway presented was tentative.



shifted downfield 0.1 ppm compared to that of **1**, as was observed for the corresponding leucomycin analogues[4].

It was postulated, accordingly, that antibiotic SF-837 (**1**) had the same skeleton as that of leucomycin A_6 (**2**), and that the difference came from acyl groups attached to the macrocyclic lactone. This postulation was fully supported by the detailed examination of the mass spectrum of **1** (Fig. 3a)*. The spectrum was analyzed by comparing

* To our best knowledge, there has been no report on the direct mass spectrometry of an intact macrolide antibiotic related to SF-837.

it with the spectra of leucomycins, whose structures were already established. Figures 4 and 5 show mass spectra above m/e 150 of leucomycin A_6 (**2**) and A_8 (**3**). Table 1 indicates characteristic peaks arising from fragmentations common to **1**, **2** and **3** together with elemental compositions of these peaks and mass shifts caused by deuteration of labile hydrogens. Chart 4 shows structures of principal fragment ions assessed.

Leucomycins (**2** and **3**) showed two strong peaks at m/e 173 and 174, and a weak peak at m/e 190. The elemental compositions of these peaks were $C_8H_{15}NO_3$, $C_8H_{16}NO_3$ and $C_8H_{16}NO_4$, which were consistent with mycaminose fragment ions, $\mathbf{a}_1$, $\mathbf{a}_2$ and $\mathbf{a}_3$, respectively. These fragment ions appeared as such in the spectrum of **1**. A relatively strong peak at m/e 201 appeared in the spectrum of **2**. It had an elemental composition $C_{10}H_{17}O_4$ and it was assigned to O-propionyl-mycarose fragment ion ($\mathbf{b}_2$). As was expected, this peak was not observed in the spectrum of **3**, and instead, a peak due to O-acetyl-mycarose fragment ion ($\mathbf{b}_1$) was observed at m/e 187. Fragment ions arising from the disaccharide moiety were recognized at m/e 300, 360 and 374. The peak at m/e 374 seen in the spectrum of **2** had a composition $C_{18}H_{32}NO_7$, and shifted two mass units to higher upon deuteration of labile hydrogens. Again, this peak was not present in the spectrum of **3**, where a peak shifted to lower mass by 14 units appeared at m/e 360. These data were consistent with O-propionyl and O-acetyl-mycarosyl-deoxymycaminose structures (fragment ions $\mathbf{c}_2$ and $\mathbf{c}_3$). The peak at m/e 300, which was observed in common in two macrolides had a composition $C_{15}H_{26}NO_5$, supporting the deacyloxy disugar structure $\mathbf{c}_1$. The spectrum of **1** showed fragment ions at m/e 201, 300 and 374. Thus, as far as sugar components were concerned, SF-837 (**1**) gave the same fragment ions as those of leucomycin A_6 (**2**), suggesting the O-propionyl sugar structure.

Fragment ions arising from macrocyclic lactones were recognized in peaks at m/e 391, 405, 409, 423, 472 and 486. In this case, leucomycins A_6 (**2**) and A_8 (**3**) gave identical fragment peaks, while SF-837 (**1**) showed peaks shifted 14 mass units upward. Thus, leucomycins gave a peak at m/e 409 which was assigned to the sixteen-membered macrocyclic lactone containing O-acetyl group at C-3 (fragment ion $\mathbf{d}_3$). The ($\mathbf{d}_3$ minus 18) peak appeared at m/e 391 ($\mathbf{d}_1$).

The corresponding lactone peaks of SF-837 (**1**) were found at m/e 423 and 405. The peak at m/e 423 had a composition $C_{23}H_{35}O_7$ and shifted to m/e 424 upon deuteration. This result, coupled with the GLC datum that two propionyl groups are present in **1**, suggests that the lactone fragment ion $\mathbf{d}_4$ probably contains a propionyl group. Dehydration of the fragment $\mathbf{d}_4$ would give a peak at m/e 405 ($\mathbf{d}_2$). It was noted in this fragmentation that the lactone fragment ions ($\mathbf{d}_1 \sim \mathbf{d}_4$) were formed exclusively by the cleavage of an aglycone-ether oxygen bond, rather than the cleavage of an ether oxygen-sugar bond that takes place frequently in the fragmentation of glycosides.

The peaks above m/e 555 in the mass spectrum of **1** had m/e values again 14 units higher than those in **2** and **3**. Thus, both **2** and **3** gave peaks at m/e 555, 583, 599, 628, 641 and 726. The corresponding peaks in **1** were found at m/e 569, 597, 613, 642, 655 and 740. Since these peaks were due to mycaminosyl-lactone fragment ions ($\mathbf{e}_1 \sim \mathbf{e}_{10}$), and mycarosyl-mycaminosyl-lactone fragment ions ($\mathbf{f}_1 \sim \mathbf{f}_2$), the difference of

14 mass units may be reasonably ascribable to the difference of acyl groups attached to the macrocyclic lactones. Each of three macrolides gave the expected molecular ions at m/e 785 ($\mathbf{g}_1$), 799 ($\mathbf{g}_2$) and 813 ($\mathbf{g}_3$).

Careful examination of mass charts of macrolides revealed that the spectra were considerably dependent on temperature of measurement. Particularly noteworthy was the charts taken above 220°C which, differed from the normal mass charts taken below 190°C, showed strong peaks arising from thermal decomposition. This is shown in Fig. 3b, where peaks due to thermal decomposition are observed at m/e 568, 666 and 739 in **1**. The former two peaks are observed also in the spectra of **2** and **3**, without any mass shift. The most intense thermal peak at m/e 666 (fragment ion $\mathbf{h}_2$) had a composition $C_{35}H_{56}NO_{11}$ and shifted 3 masses upward upon deuteration, being consistent with the structure shown. The formation of $\mathbf{h}_2$ might be explained by the release of fatty acids attached to the lactone ring from the deacyloxy fragment ions $\mathbf{f}_2$ or $\mathbf{f}_1$. Another thermal peak at m/e 568 ($\mathbf{h}_1$) was interpreted similarly by the loss of fatty acids from fragment ions $\mathbf{e}_8$ and $\mathbf{e}_7$*. As described later, the fragmentations discussed above were supported by the mass spectra of derivatives of SF-837 and leucomycins.

On the basis of these results, it was increasingly clear that SF-837 (**1**) consisted of the same framework as that of leucomycins with two propionyl groups: one at C-4″ of mycarose and the other at C-3 of macrocyclic lactone. A propionyl group on the mycarose moiety was selectively cleaved as a propionyloxy radical by electron impact, and another propionyl group on the lactone ring was easily removed by heat as a propionic acid, yielding the skeleton fragment ion $\mathbf{h}_2$ identical with that from the leucomycins by the same processes**.

Chemical Studies

The structure of antibiotic SF-837 (**1**) deduced by the physico-chemical methods was confirmed by the chemical degradation studies as summarized in Charts 5 and 6.

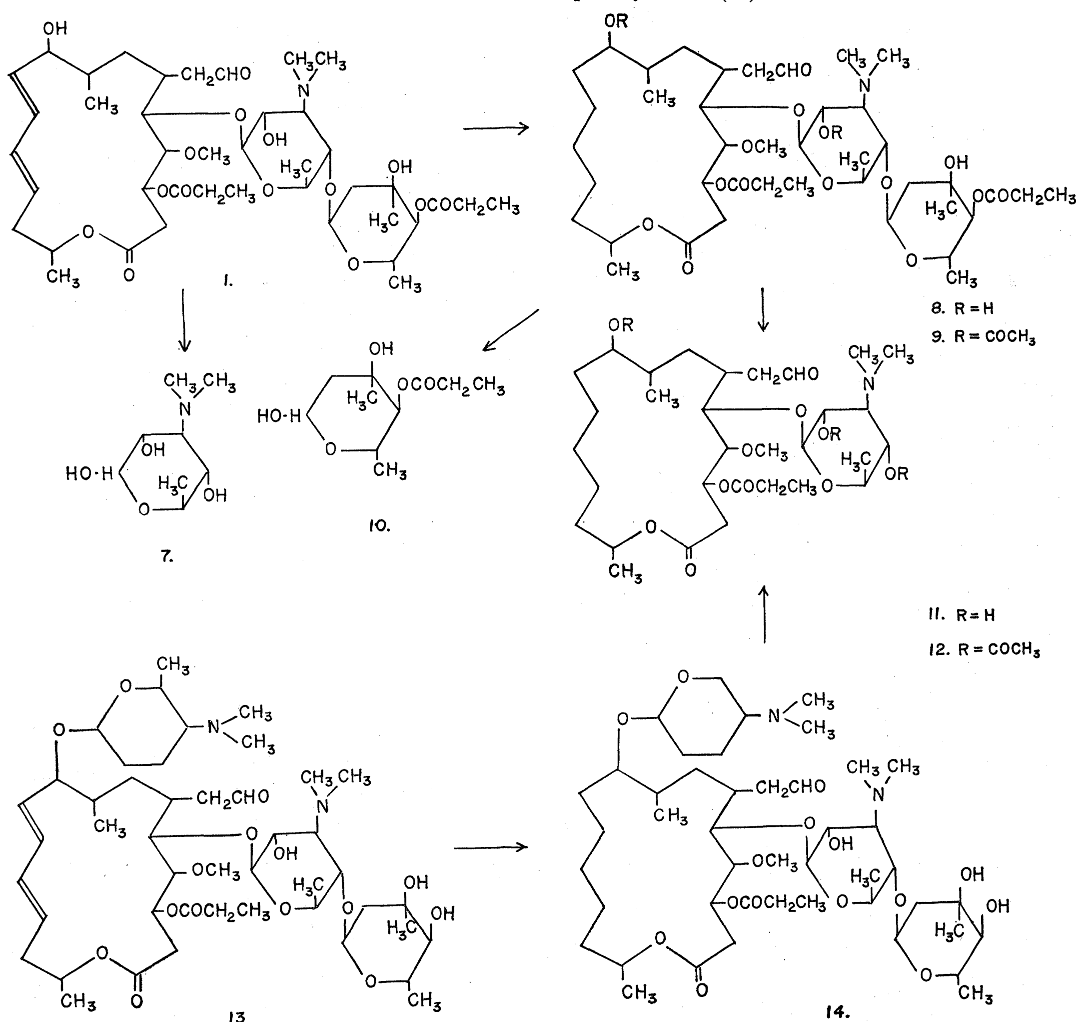
Vigorous acid hydrolysis of **1** with 3 N hydrochloric acid at 100°C for 2 hours, followed by chromatographic purification over Dowex 50W×2 (H^+) resin yielded crystalline aminosugar (**7**) hydrochloride with melting point of 120~124°C. This was identified as mycaminose hydrochloride by a direct comparison with an authentic sample obtained from leucomycins[5]. Both compounds gave identical melting points, IR and mass spectra, and $[\alpha]_D$ values.

Catalytic reduction of **1** over Pd-carbon gave, after absorption of 2 moles of hydrogen, tetrahydro derivative (**8**). It afforded crystalline di-O-acetate (**9**). Both **8** and **9** showed no UV band at 232 mμ, indicating saturation of the conjugated double bond. The NMR spectrum of the diacetyl derivative (**9**) exhibited the presence of an aldehyde proton at δ 9.61 and disappearance of olefinic protons. Mild acid hydro-

* There is another possible route for $\mathbf{h}_1$ and $\mathbf{h}_2$ via fragment ion $\mathbf{h}_3$, *i. e.*, first removing fatty acids on the lactone rings followed by releasing acyloxy groups on the mycarose moiety.

** It is generally recognized that mass spectrometry is often insensitive to the stereochemistry. Therefore, the fragmentation data alone do not imply that the steric configuration of SF-837 is identical with that of leucomycins.

Chart 5. Chemical degradation of antibiotic SF-837 (**1**) and its correlation with spiramycin III (**13**).



lysis of the tetrahydro derivative (**8**) with 0.3 N hydrochloric acid at room temperature for 20 hours, followed by ether extraction at pH 4 afforded a neutral sugar fraction. Chromatographic purification over silica gel and evaporation of the fractions containing the neutral sugar gave a white solid, which was crystallized from benzene. m.p. 110~112°C. The NMR spectrum of the neutral sugar (**10**) in $CDCl_3$ indicated propionyl signals at δ 2.45 and 1.19, together with a broad singlet at δ 5.22 (H_1''), a doublet at δ 4.68 (H_4''), two quartets at δ 4.22 (H_5''), a multiplet at 2.0 (H_2'') and a broad singlet at 3.05 (OH''). Compound **10** was identified as 4-O-propionyl-mycarose[3], by comparing it with an authentic sample prepared from spiramycin I via tri-O-propionyl-derivative. Melting points, $[\alpha]_D$, IR, NMR and mass spectra of both compounds were completely identical. Crystalline **10** showed mutarotation in water, $[\alpha]_D$ $-78° \rightarrow -60°$. Since methyl 4-O-propionyl α-L-mycaroside showed $[\alpha]_D$ $-145°$, and the β-L-anomer $[\alpha]_D$ $+20°$ [3]; α-L-form was suggested for **10** obtained first as crystals.

The mild acid hydrolyzate of tetrahydro SF-837 (**8**), remaining after the ether

extraction of the neutral fraction at pH 4, was adjusted to pH 9, and extracted with chloroform. Chromatographic purification of the chloroform extract over silica gel gave demycarosyl-tetrahydro SF-837 (**11**) as white powder. $[\alpha]_D$ −13°. Its NMR spectrum shown in Fig. 6 revealed a dimethylamino signal at δ 2.5, an anomeric proton at δ 4.5, an aldehyde at δ 9.63 and a methoxyl at δ 3.53, but no signals assignable to the mycarose moiety. Treatment of **11** with acetic anhydride-pyridine gave tri-O-acetyl-demycarosyl-tetrahydro SF-837 (**12**). Compound **11** was identified by comparison with tetrahydroforocidine C*, which was prepared from spiramycin III (**13**) via tetrahydro derivative (**14**). Tetrahydroforocidine C showed $[\alpha]_D$ −10°, and identical IR, NMR and mass spectra and Rf values on TLC with those of demycarosyl-tetrahydro SF-837 (**11**). These results proved that antibiotic SF-837 (**1**) consisted of mycaminose, 4″-O-propionyl-mycarose, and a sixteen-membered macrocyclic lactone containing a propionyl group at C-3, and that mycaminose was attached to the lactone in the same way as that of the spiramycins and the leucomycins. The remaining problem to be chemically proved was the linkage between the mycarose and mycaminose moieties. This was resolved as shown in Chart 6.

Chart 6. Chemical correlation of antibiotic SF-837 (**1**) and leucomycins (**15**).

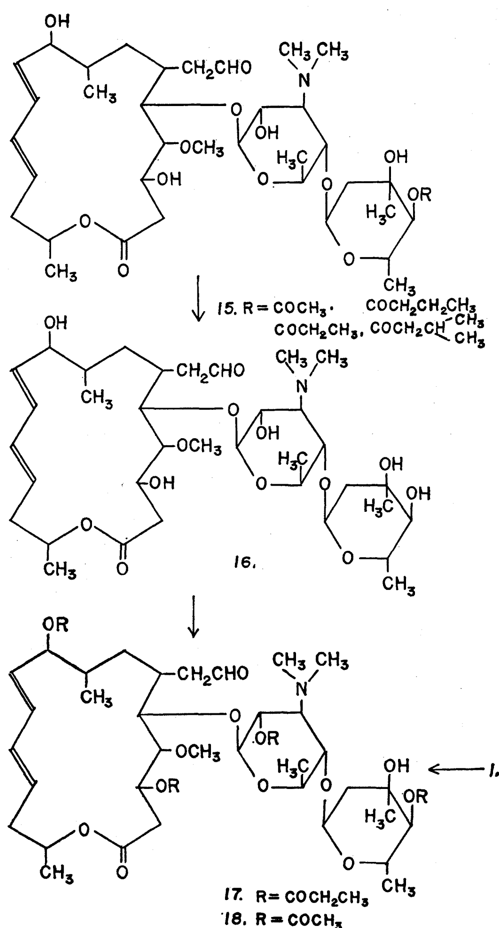
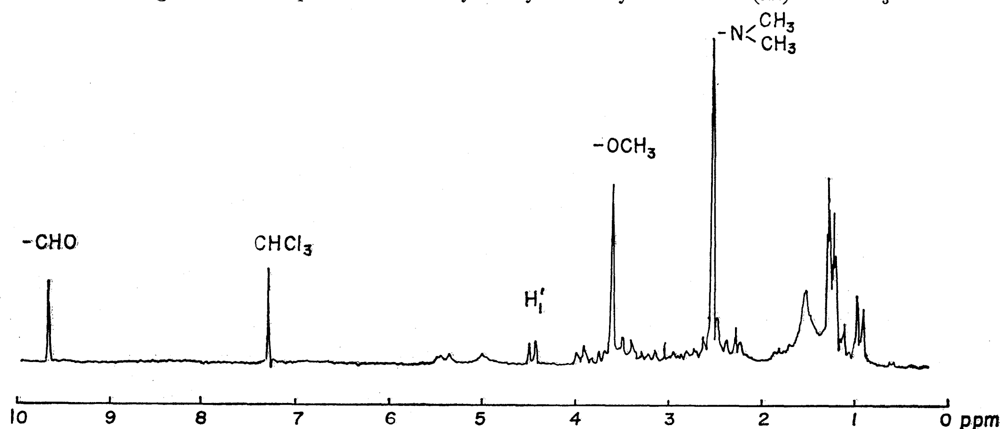
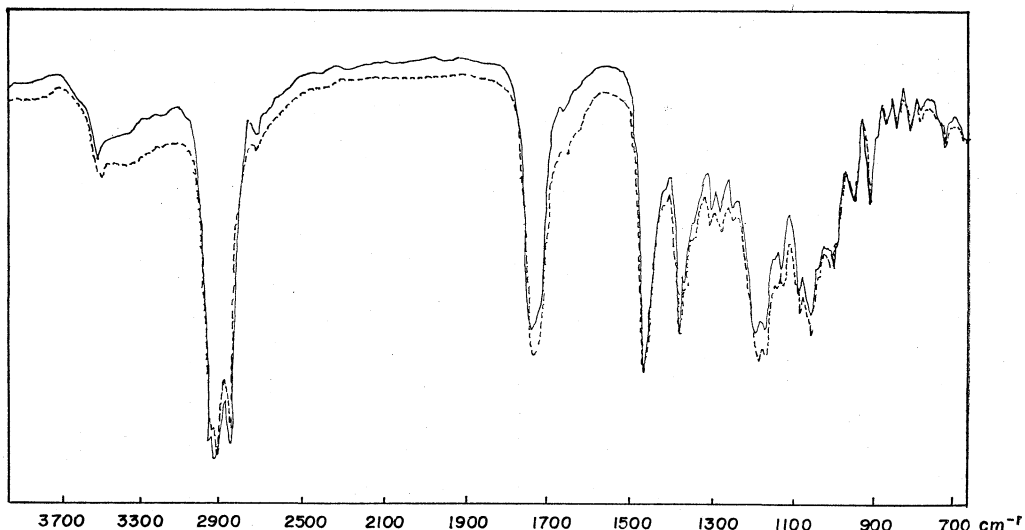


Fig. 6. NMR spectrum of demycarosyl-tetrahydro SF-837 (**11**) in $CDCl_3$.



* This compound was named according to notations proposed by ŌMURA and coworkers[6].

Fig. 7. IR spectra of tri-O-propionyl leucomycin A_7 (——) and di-O-propionyl SF-837 (**17**) (----) in nujol.



According to ŌMURA and coworkers[7], a commercial preparation of the leucomycins was separated by alumina chromatography into a leucomycin Fr group (A_1, A_5, A_7 and A_9) (**15**) and a leucomycin Ac group (A_3, A_4, A_6 and A_8). The former was treated with *Mucor spinescens* to hydrolyze acyl groups attached to the mycarose moiety. The deacylated product (**16**) was acetylated with acetic anhydride in pyridine at room temperature to give the tetra-O-acetate (**6**), which was identified as di-O-acetyl leucomycin A_8 [3], thereby confirming the structure of **16**. Treatment of **16** with propionic anhydride in pyridine at 28°C for 7 days, followed by purification by TLC gave crystalline tri-O-propionyl leucomycin A_7, which was identical with di-O-propionyl SF-837 (**17**) prepared from **1** in all respects of melting points, $[\alpha]_D$, Rf values on TLC, and IR and mass spectra. Fig. 7 illustrates IR spectra of both compounds. Since all steric configurations of the leucomycins have been resolved[8], the above results complete the determination of the stereochemistry of SF-837 (**1**) including its absolute configuration.

Mass Spectra of Derivatives of SF-837 and Leucomycins

Table 2 summarized mass fragments of tetrahydro SF-837 (**8**), demycarosyl-tetrahydro SF-837 (**11**), di-O-acetyl SF-837 (**4**), di-O-propionyl SF-837 (**17**) and related compounds. The spectrum of tetrahydro derivative (**8**) was essentially that of SF-837 (**1**), except for up-shifting of 4 masses of the fragment ions containing macrocyclic lactone (**d**, **e**, **f** and **g** series). The mass spectra of di-O-acyl SF-837 (**4** and **17**), and di-O-acetyl leucomycin A_6 (**5**) and A_8 (**6**) could be interpreted on the basis of the fragmentation proposed for the parent macrolides (**1**, **2** and **3**). The mycarose fragment ions $\mathbf{b}_1$ and $\mathbf{b}_2$ were commonly recognized in the spectra of di-acyl derivatives, while the mycaminose (**a** series) and mycarosyl-mycaminose fragment ions (**c** series) showed increment of 42 or 56 mass units, owing to an acetyl or a propionyl group introduced

Table 2. Mass fragment ions of tetrahydro SF-837 (**8**), demycarosyl-tetrahydro SF-837 (**11**), diacetyl SF-837 (**4**), dipropionyl SF-837 (**17**), diacetyl leucomycin A_6 (**5**), diacetyl leucomycin A_8 (**6**) and deacylated leucomycin (**16**)

Fragment ion	Nominal mass (m/e) and relative intensity *						
	8	**11**	**4**	**17**	**5**	**6**	**16**
$\mathbf{a}_1$ minus fatty acid			155 s	(156 s)	155 s	155 s	
$\mathbf{a}_1$	173 s	173 s	215m				173 s
$\mathbf{a}_2$	174 s	174 s	216 s	230 s	216m	216m	174 s
$\mathbf{a}_3$	190m	190 s					190 s
$\mathbf{b}_2$ ($\mathbf{b}_1$)	201 s		201 s	201m	201m	187m	145m
$\mathbf{c}_1$	300w		342m	356w	342m	342m	
$\mathbf{c}_3$ ($\mathbf{c}_2$)	374m		416 s	430m	416 s	402 s	318m
$\mathbf{d}_2$	409w	409w					349m
$\mathbf{d}_4$ ($\mathbf{d}_3$) minus fatty acid			405w	405w	391w	391w	
$\mathbf{d}_4$ ($\mathbf{d}_3$) minus CO	399w		437m	451w	423m	423m	
$\mathbf{d}_4$ ($\mathbf{d}_3$)	427m	427m	465w	479w	451w	451w	367m
$\mathbf{d}_4$ ($\mathbf{d}_3$) plus 63	490m	490m					430w
$\mathbf{e}_2$ ($\mathbf{e}_1$)	573w	589w	653m	681m	639 s	639 s	513w
$\mathbf{e}_4$ ($\mathbf{e}_3$)	601w		681w	709w	667w	667w	541w
$\mathbf{e}_6$ ($\mathbf{e}_5$) minus CO				697 s			529m
$\mathbf{e}_6$ ($\mathbf{e}_5$) minus 1	616w		696w	724w	682w	682w	557m
$\mathbf{e}_6$ ($\mathbf{e}_5$)	617w			725w			
$\mathbf{e}_8$ ($\mathbf{e}_7$)	646m		726w	754w	712w	712w	586w
$\mathbf{h}_2$	670w		750w	778m	750w	750m	
$\mathbf{f}_2$ ($\mathbf{f}_1$)	744 s		824m	852m	810 s	810 s	
g minus CO			869w	897w	855w	841w	
g	817w	617m	897w	925w	883w	869w	711w

* s : strong m : medium w : weak

newly at C-2′ of the mycaminose moiety. Similarly, lactone fragment ions (**d** series) and sugar-lactone fragment ions (**e** and **f** series) showed increment of one acyl group (42 or 56), and two acyl groups (84 or 112), respectively. Thus, the fragmentation proposed for the free macrolides was corroborated by the substituent-labeled compounds.

One of the most principal fragmentations in the spectra of macrolides, was ready loss of an acyloxy group attached to C-4″ of the mycarose moiety. This seemed to be characteristic to macrolides themselves, since methyl 4-O-propionyl-mycaroside gave no (M^+ minus propionyloxy) peak, but a weak (M^+ minus propionic acid) peak[3]. In contrast, a (M^+ minus acyloxy) peak was the base peak in all the spectra above m/e 150 of compounds **1**, **2**, **3** and **8**. This fragment ion (**f** series) was also relatively strong in the di-O-acyl derivatives (**4**, **5**, **6** and **17**), suggesting that the acyloxy group on the mycarose moiety was released more easily than the other acyloxy groups attached to mycaminose and macrocyclic lactones. In this connection, mass spectrum of the compound **16** was of special interest, since it contained no acyl group as the side chain. Here, formation of a fragment ion **f** did not occur to any significant extent. Other fragment ions characteristic to the leucomycins were all recognized, except for fragment ion $\mathbf{c}_1$ that was originated from **f**.

In conclusion, the structure of antibiotic SF-837 was elucidated as **1***.

Note added in proof: After completion of this work, SUZUKI and coworkers reported the structures of antibiotic YL-704 A and B, of which YL-704 B had the same structure as that of antibiotic SF-837 (SUZUKI, M.; I. TAKAMORI, A. KINUMAKI, Y. SUGAWARA & T. OKUDA: Tetrahedron Letters, No. 5, 435~438, 1971).

Experimental

General methods

Melting points were determined with a Yamato MT-1 melting point apparatus, and are uncorrected. Specific rotations were determined in a 10-mm cell with a Jasco Model DIP-SL automatic polarimeter. IR Spectra were measured with a Koken 401 grating spectrometer. UV Spectra were measured with a Hitachi EPS-2 recording spectrometer. NMR Spectra were measured at 100 MHz with a JNM-4H-100 spectrometer. Chemical shifts were given on the δ scale, and unless otherwise stated, all spectra were measured at *ca.* 30°C, with TMS as the internal standard. Spectra were analyzed on a first order basis.

TLC was performed with silica gel (Camag, Switzerland) activated at 105°C, as the adsorbent. Unless otherwise stated, the adsorbent thickness was 0.25 mm, and indication was effected with sulfuric acid. Preparative TLC was carried out with silica gel admixed with fluorecent agents.

Mass spectra

The low resolution mass spectra were recorded on a JMS-01SG high resolution mass spectrometer using the direct inlet system, at an ionizing potential of 75 eV, an ionizing current of 200 μA, and a resolution of approximately 1,000 (10 % valley). High-resolution measurements were determined under the same conditions, but with a resolution of *ca.* 20,000 (10 % valley). The exact mass data were obtained by the photographic recording. All values were within ± 2 millimass units below m/e 400, and ± 9 millimass units above m/e 400 of the theoretical values calculated for elemental compositions.

Since relative peak heights were extremely sensitive to the temperature of evaporation, it was difficult to obtain the pattern coefficients with good reproducibility, particularly when scanned over wide mass range. Relative abundances shown in Figs. 3~5 were compared on the peaks above m/e 150, and have deviation of 30 % at a maximum.

Deuterium-labeled SF-837 was prepared by dissolving SF-837 in a mixture of pyridine and D_2O followed by evaporation of solvents *in vacuo*. To minimize partial reexchange of deuterium in the ion source, the deuterated compound was dissolved in acetone-D_2O, and packed in a glass capillery for analysis. The percentage of the total deuterium incorporation as judged from relative peak heights amounted to 60 %.

Isolation of mycaminose (3, 6-dideoxy-3-dimethylamino-D-glucopyranose) (**7**) from SF-837 (**1**)

A solution of **1** (870 mg) in 3 N hydrochloric acid (13 ml) was refluxed for 2 hours. Dark brown materials precipitated during the reaction was removed by filtration, and the filtrate and washings were combined, and extracted twice with chloroform (each 30 ml), and one time with *n*-butanol (30 ml). The remaining aqueous layer was concentrated to a brown syrup (180 mg). This was dissolved in water (3 ml), and applied to a column of Dowex 50 W×2 (H^+, 20 ml). After washing with water, the column was eluted with 0.5 N hydrochloric acid. The fractions that gave positive anthrone test were collected and

* According to the naming of Chemical Abstracts, SF-837 was named 7-(formylmethyl)-4,10-dihydroxy-5-methoxy-9,16-dimethyl-2-oxooxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-O-(2,6-dideoxy-3-C-methyl-α-L-ribohexopyranosyl)-3-(dimethylamino)-β-D-glucopyranoside 4,4-dipropionate (ester).

evaporated to a colorless syrup (55 mg). Crystallization from 90 % *iso*-propanol yielded white needles of **7** hydrochloride (32 mg). m. p. 120~124°C, $[\alpha]_D^{22}$ +32° (*c* 1, methanol).

Anal. Calcd. for $C_8H_{17}NO_4 \cdot HCl \cdot H_2O$: C 39.10, H 8.20, N 5.70 %.
Found : C 39.38, H 8.02, N 5.65 %.

On silica gel thin-layer plates, it showed Rf 0.2 with *n*-butanol - methanol - water (4 : 1 : 2), and Rf 0.3 with *n*-butanol - acetic acid - water (3 : 1 : 1).

Preparation of tetrahydro SF-837 (**8**)

A solution of **1** (1.0 g) in ethanol (30 ml) was reduced over Pd-carbon (200 mg), until 1.97 moles of hydrogen were absorbed (2.5 hours). The reaction mixture was filtered, and the filtrate was evaporated to dryness. Tetrahydro SF-837 (**8**) was obtained as white powder (920 mg), m. p. 108°C, $[\alpha]_D^{20}$ −57° (*c* 1, ethanol).

Anal. Calcd. for $C_{41}H_{71}NO_{15}$ (MW, 817): C 60.22, H 8.69, N 1.71 %.
Found : C 59.74, H 9.14, N 1.68 %.

Preparation of di-O-acetyl-tetrahydro SF-837 (**9**)

To a solution of tetrahydro SF-837 (**8**) (180 mg) in pyridine (1 ml) was added 0.4 ml of acetic anhydride, and the mixture stood at room temperature overnight, and then poured into ice-water (15 ml) under stirring. White solid precipitated was taken up by filtration, washed with water and dried *in vacuo* to afford white powder (190 mg). This was dissolved in carbon tetrachloride and crystallized upon standing. White needles of **9** (135 mg), m. p. 110~113°C, $[\alpha]_D^{20}$ −54° (*c* 1, ethanol). It showed no UV maximum in ethanol.

Anal. Calcd. for $C_{45}H_{75}NO_{17}$ (MW, 901): C 59.93, H 8.32, N 1.55 %.
Found : C 59.74, H 8.83, N 1.93 %.

Isolation of 4-O-propionyl-mycarose (**10**) from tetrahydro SF-837 (**8**)

A solution of **8** (600 mg) in 0.3 N hydrochloric acid (15 ml) was kept at room temperature for 20 hours. After addition of water (50 ml), the solution was adjusted to pH 4, and extracted twice with ethyl ether (each 60 ml). The ether layer was washed with water, dried over sodium sulfate and evaporated to white solid (120 mg). This was dissolved in benzene (5 ml), and chromatographed on a silica gel column (20 ml), developing with benzene - ethyl acetate (1 : 1). The fractions showing positive vanilline perchloric acid test were collected and evaporated to crystalline residue, which was recrystallized from benzene. 4-O-Propionyl-mycarose (**10**) was obtained as white needles, 72 mg, m. p. 110~112°C, $[\alpha]_D^{20}$ −78° (after 5 minutes)→ −60° (after 24 hours) (*c* 1, water).

Anal. Calcd. for $C_{10}H_{18}O_5$ (MW, 218): C 55.05, H 8.26 %.
Found : C 55.31, H 8.25 %.
Mass fragment ions: M^+ (m/e 218), $M^+ -1$ (m/e 217), $M^+ -OH$ (m/e 201), $M^+ -75$ (m/e 143), propionium ion (m/e 57, base peak).

Isolation of demycarosyl-tetrahydro SF-837 (**11**)

After ether extraction as described above, the aqueous acidic solution was adjusted to pH 9, and extracted twice with chloroform (60 ml each). The chloroform layer was washed, dried over sodium sulfate, and evaporated to dryness to give white powder (415 mg). This was dissolved in ethyl acetate (4 ml), and chromatographed on a silica gel column (30 ml), washing with ethyl acetate (50 ml), and developing with a mixture of ethyl acetate - methanol (1 : 1). The effluents contained **11** were collected and concentrated to dryness to yield white powder of demycarosyl-tetrahydro SF-837 (**11**) (387 mg), m. p. 106 ~ 108°C, $[\alpha]_D^{20}$ −13° (*c* 1, ethanol). It showed no UV absorption maximum in ethanol.

Anal. Calcd. for $C_{31}H_{55}NO_{11}$ (MW, 617): C 60.29, H 8.91, N 2.27 %.
Found : C 58.70, H 8.96, N 2.23 %.
Rf Values on silica gel plates: 0.56 with methanol, 0.18 with chloroform - methanol (1 : 1).

Preparation of tri-O-acetyl-demycarosyl-tetrahydro SF-837 (**12**)

A mixture of demycarosyl-tetrahydro SF-837 (**11**) (200 mg), acetic anhydride (0.6 ml) and pyridine (2 ml) was kept at room temperature for 20 hours, and poured into ice-water (20 ml). White solid (240 mg) precipitated was taken up, washed with water, and dried. This was dissolved in benzene (3 ml), and chromatographed on a silica gel column (20 ml), developing with benzene. Evaporation of the effluents that contained **12** gave white powder of tri-O-acetyl-demycarosyl-tetrahydro SF-837 (**12**) (190 mg). m.p. 95~98°C, $[\alpha]_D^{20}$ −12° (*c* 1, ethanol). Its NMR spectrum in $CDCl_3$ is shown in Fig. 6.

Anal. Calcd. for $C_{37}H_{57}NO_{14}$ (MW, 739): C 60.08, H 7.70, N 1.89 %.
Found : C 59.40, H 8.44, N 1.94 %.

Preparation of tetrahydroforocidine C (**11**) from spiramycin III (**13**)

A solution of **13** (1.3 g) in ethanol (50 ml) that was prepared from commercial sample of the spiramycins by the use of silica gel column chromatography was shaken with Pd-carbon (250 mg) for 2 hours, during which time 1.98 moles of hydrogen were absorbed. The catalyst was removed by filtration, and the filtrate was concentrated to give tetrahydro spiramycin III (**14**) as white powder (1.2 g), m. p. 120~123°C.

The compound **14** (1.1 g) was dissolved in 0.3 N hydrochloric acid (15 ml), and heated at 65°C for 1 hour. To the reaction mixture was added 50 ml of water, and extracted twice with chloroform (60 ml each) to remove the mycarose portion. The remaining aqueous solution was adjusted to pH 4.0, and extracted twice with ethyl ether (60 ml each). After washing with water and drying over sodium sulfate, the ether layer was evaporated to crude powder (500 mg). The forosamine portion was remained in aqueous layer. The crude powder of the ether extract was dissolved in a mixture of benzene - acetone (5 : 1) (10 ml), and chromatographed on a silica gel column (30 ml), developing with the same solvent mixture. Evaporation of the effluents that contained **11** gave white powder of tetrahydroforocidine C (**11**) (350 mg). m. p. 105~106°C, $[\alpha]_D^{22}$ −10° (*c* 1, ethanol).

Anal. Found : C 60.00, H 8.96, N 2.24 %.

Preparation of di-O-propionyl SF-837 (**17**)

A mixture of **1** (180 mg) and propionic anhydride (0.5 ml) in pyridine (5 ml) was shaken in a sealed tube at 28°C for 5 days. The reaction mixture was concentrated to dryness, and the residue was dissolved in chloroform. After washing with water and drying over sodium sulfate, the chloroform layer was evaporated to white powder (210 mg). It showed a major spot of Rf 0.6 and a minor of Rf 0.5 on silica gel TLC developed with benzene - acetone (5 : 1). The former was separated from the latter by preparative silica gel plates, using the same solvent system mentioned above. The main band containing **17** was extracted with methanol, and the extracts were evaporated to white powder (95 mg). Crystallization from carbon tetrachloride gave colorless needles of di-O-propionyl SF-837 (**17**, 60 mg). m. p. 109~114°C, $[\alpha]_D^{22}$ −75° (*c* 0.7, chloroform). Its IR spectrum is illustrated in Fig. 8.

Anal. Calcd. for $C_{47}H_{75}NO_{17}$ (MW, 951): C 60.95, H 8.16, N 1.51 %.
Found : C 60.82, H 8.02, N 1.46 %.

Rf Value on silica gel TLC : 0.6 with benzene - acetone (5 : 1). Melting point, Rf values on TLC, IR and mass spectra of **17** were identical with those of tri-O-propionyl leucomycin A_7.

Preparation of deacylated leucomycin (**16**)

A solution of the leucomycin Fr group (**15**) (100 mg) in acidic water (6 ml, pH 3) was added to a broth of *Mucor spinescens* IAM 6071, which, prior to the addition of the substrate, was shaken at 28°C for 70 hours in a medium containing 2.0 % sucrose, 1.0 % glucose, 1.0 % peptone, 1.0 % Soybean meal and 1.0 % Pharmamedia (pH 7). The culture was continued for another 40 hours. The culture broth (300 ml) was filtered, and wet mycelia were washed with water and extracted with 50 ml of methanol. Evaporation of methanol from the extracts gave aqueous solution, which was combined with broth filtrate and washings.

The solution was adjusted to pH 9, and extracted twice with ethyl acetate (80 ml each). The ethyl acetate layer was washed with water, dried over sodium sulfate, and evaporated to dryness to give a crude powder (50 mg). This was dissolved in benzene, and chromatographed on a silica gel column (30 ml), developing with benzene - acetone (4 : 1). The effluents that contained **16** were evaporated to white powder of deacylated leucomycin (**16**), m. p. 65～70°C (sintered), with decomposition above 230°C. $[\alpha]_D^{21}$ −58° (*c* 1, ethanol). It showed a UV maximum at 232 mμ ($E_{1cm}^{1\%}$ 310) in ethanol. NMR Spectrum of **16** in $CDCl_3$ showed the following signals, C$\underline{H}$O at δ 9.6, OC$\underline{H}_3$ at δ 3.5 and N(C$\underline{H}_3$)$_2$ at δ 2.5, but no acetyl signal.

Anal. Calcd. for $C_{35}H_{59}NO_{13}$ (MW, 701): C 59.90, H 8.47, N 2.00 %.
Found : C 59.46, H 9.01, N 1.93 %.

Preparation of tri-O-propionyl leucomycin A_7 (**17**) from deacylated leucomycin (**16**)

A mixture of **16** (320 mg), propionic anhydride (0.8 ml) and pyridine (5 ml) was shaken in a sealed tube at 28°C for 7 days. Excess of pyridine was removed by evaporation, and the residue was dissolved in chloroform (30 ml). The chloroform layer was washed three times with water (15 ml each), dried over sodium sulfate and evaporated to white powder (360 mg). Its silica gel TLC developed with benzene - acetone (5 : 1) showed a major spot of Rf 0.6, together with minor spots of Rf 0.55, 0.5 and 0.3. The major product of Rf 0.6 was separated by the use of preparative TLC developed four times with benzene - acetone (10 : 1). Extraction of a main band with acetone followed by evaporation of solvent gave white powder, which was crystallized from carbon tetrachloride. Tri-O-propionyl leucomycin A_7 (**17**) was obtained as colorless needles, 65 mg, m. p. 110～115°C, $[\alpha]_D^{22}$ −74° (*c* 1, chloroform).

Anal. Calcd. for $C_{47}H_{75}NO_{17}$ (MW, 925): C 60.95, H 8.16, N 1.51 %.
Found : C 61.28, H 8.26, N 1.63 %.

Preparation of di-O-acetyl leucomycin A_8 (**6**) from deacylated leucomycin (**16**)

A mixture of **16** (70 mg), acetic anhydride (0.15 ml) and pyridine (1.5 ml) was stood at room temperature for 2 days, and heated at 55°C for 16 hours. The reaction mixture was evaporated to dryness, and the residue was dissolved in ethyl acetate. After washing with water, the organic solvent layer was concentrated, and applied on a silica gel thin-layer plate, which was developed first with benzene - acetone (3 : 1), and then twice with benzene - acetone (9 : 1). The acetone extracts from a main band were evaporated to give white powder of di-O-acetyl leucomycin A_8 (**6**) (23 mg), which was crystallized from carbon tetrachloride, m. p. 134～137°C.

Anal. Calcd. for $C_{43}H_{67}NO_{17}$ (MW, 869): C 59.37, H 7.76, N 1.61 %.
Found : C 60.08, H 7.50, N 1.56 %.

Authentic di-O-acetyl leucomycin A_8 prepared from leucomycin A_8 showed m. p. 130～133°C, and m. p. 130～135°C when admixed with **6**. Both compounds exhibited identical IR and mass spectra.

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