STUDIES ON ANTIBIOTIC SF-837, A NEW ANTIBIOTIC

IV. STRUCTURES OF ANTIBIOTICS SF-837 A_2, A_3 AND A_4

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(Received for publication March 26, 1971)

The present paper presents evidence as to the structures of antibiotics SF-837 A_2, A_3 and A_4, minor components of an antibiotic mixture produced by *Streptomyces mycarofaciens*. Mass spectrometry of three new macrolides, antibiotics SF-837 A_2, A_3 and A_4, revealed that each is composed of the elements of mycaminose, an O-acyl mycarose and an O-acyl macrocyclic lactone. Microbiological deacylation was employed to correlate antibiotic SF-837 A_2 with the parent SF-837. Manganese dioxide oxidation of antibiotics SF-837 and SF-837 A_2 yielded compounds identical with SF-837 A_3 and A_4, respectively. These transformations define the structure and stereochemistry of the three minor components of the SF-837 antibiotic mixture.

Antibiotics SF-837 A_2, A_3 and A_4 are minor components of a mixture produced by *Streptomyces mycarofaciens*[1]. Substance SF-837*, the major component of the mixture, is a new macrolide antibiotic of the leucomycin type (cf. Part II of this series[2]). The present paper presents evidence as to the structures of antibiotics SF-837 A_2, A_3 and A_4.

Structure of SF-837 A_2

In Part III of this series[1], it was mentioned that antibiotic SF-837 A_2 is closely related to SF-837 as evidenced by their similar UV and IR spectra and $[\alpha]_D$ values. The similarity between these two macrolides was further supported by the NMR spectrum of SF-837 A_2 (Fig. 1), which exhibited all the salient features of the spectrum of SF-837, including an aldehyde proton (δ 9.64), four olefinic proton (5.5~6.8), anomeric proton (5.10, 4.45), methoxyl proton (3.56) and dimethylamino proton (2.54) signals.

The molecular formula of SF-837 A_2 ($C_{42}H_{69}NO_{15}$) contained one extra methylene group compared with that of SF-837 ($C_{41}H_{67}NO_{15}$), and this difference was ascribed to the difference in acyl groups attached to the main framework. This was evidenced by the liberation of a molecule of *n*-butyric acid and a molecule of propionic acid from SF-837 A_2, whereas SF-837 yielded two molecules of propionic acid on hydrolysis. Thus, SF-837 A_2 is postulated as a homologue of SF-837, in which either one of the

* The structure of SF-837 (**1**) appears to be identical to that of YL-704 B reported by SUZUKI and coworkers (Tetrahedron Letters 1971-5 : 435~438, 1971).

Fig. 1. NMR spectrum of antibiotic SF-837 A_2 (**2**) in $CDCl_3$.

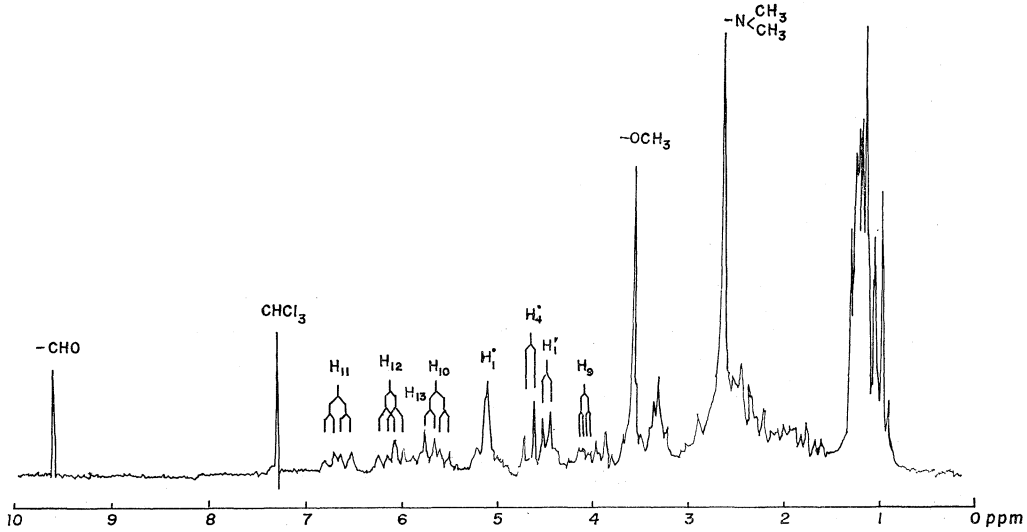
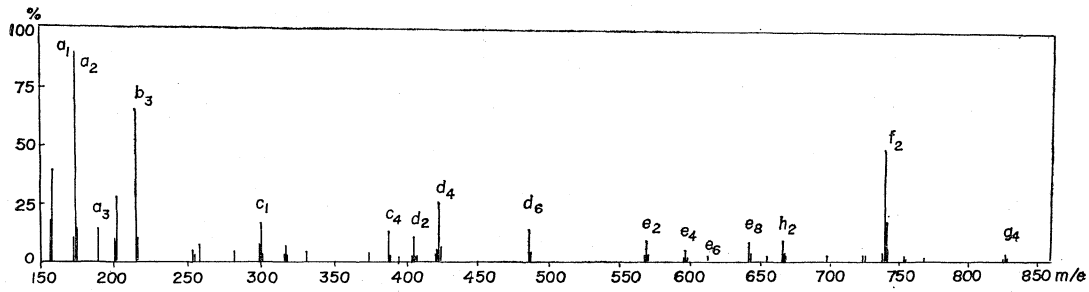


Fig. 2. Mass spectrum of antibiotic SF-837 A_2 (**2**).



two propionyl groups located at C-3 of the macrocyclic lactone and C-4″ of the mycarose moiety of SF-837 is replaced by an *n*-butyryl group. The two alternative structures were easily distinguished by mass spectrometry, based on the fragmentation pattern of SF-837.

Fig. 2 shows the mass spectrum of SF-837 A_2. When it was compared with the mass chart of SF-837 reported in Part II of this series[2], it was apparent that both spectra were essentially identical with the exception of the fragment ions derived from the O-acyl mycarose portion. Thus, SF-837 A_2 showed peaks at m/e 173, 174 and 190 assignable to the mycaminose fragment ions $\mathbf{a}_1$, $\mathbf{a}_2$ and $\mathbf{a}_3$. Lactone fragment ions $\mathbf{d}_2$, $\mathbf{d}_4$ and $\mathbf{d}_6$ were recognized at m/e 405, 423 and 486, respectively. Likewise, mycaminosyl lactone fragment ions $\mathbf{e}_2$, $\mathbf{e}_4$, $\mathbf{e}_6$ and $\mathbf{e}_8$ appeared at m/e 569, 597, 613 and 642. These results indicated that the macrocyclic lactone of SF-837 A_2 bears a propionyl group, as does that of SF-837. On the other hand, a mycarose fragment ion $\mathbf{b}_3$, a mycarosyl mycaminose fragment ion $\mathbf{c}_4$ and a molecular ion $\mathbf{g}_4$ were observed at m/e 215, 388 and 827, and they are 14 mass units higher than the corresponding peaks from SF-837. This suggested that an *n*-butyryl group is attached to the mycarose moiety in SF-837 A_2. A strong peak at m/e 740 ($\mathbf{f}_2$) together with a medium

peak at m/e 300 ($\mathbf{c}_1$) were indicative of a preferred loss of an *n*-butyryloxy group attached to C-4″ of the mycarose moiety[2]. Removal of a propionic acid residue from $\mathbf{f}_2$ would yield a peak at m/e 666 ($\mathbf{h}_2$), which was commonly observed in the spectra of SF-837 and the leucomycins.

Analysis of the mass spectrum of di-O-acetyl SF-837 A_2 led to the same conclusion. As was discussed with respect to the fragmentation pattern of di-O-acetyl SF-837[2], a mycaminose fragment ion $\mathbf{a}_2$ and a lactone fragment ion $\mathbf{d}_4$ each showed an increment of m/e 42 mass units due to the newly introduced acetyl group. The fragment ions containing two acetyl groups ($\mathbf{e}_2$, $\mathbf{e}_4$, $\mathbf{e}_6$, $\mathbf{e}_8$, $\mathbf{f}_2$ and $\mathbf{g}_4$) showed double the increment, *i.e.*, 84 mass units. In contrast, a mycarose fragment ion $\mathbf{b}_3$ was unchanged, indicating no acetyl substitution on the mycarose moiety. Thus, primarily on the basis of mass spectrometry, the structure **2** was proposed for SF-837 A_2.

Chart 1. Structures of antibiotic SF-837 (**1**) and A_2 (**2**).

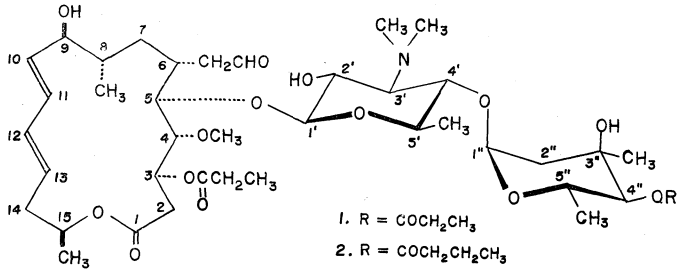
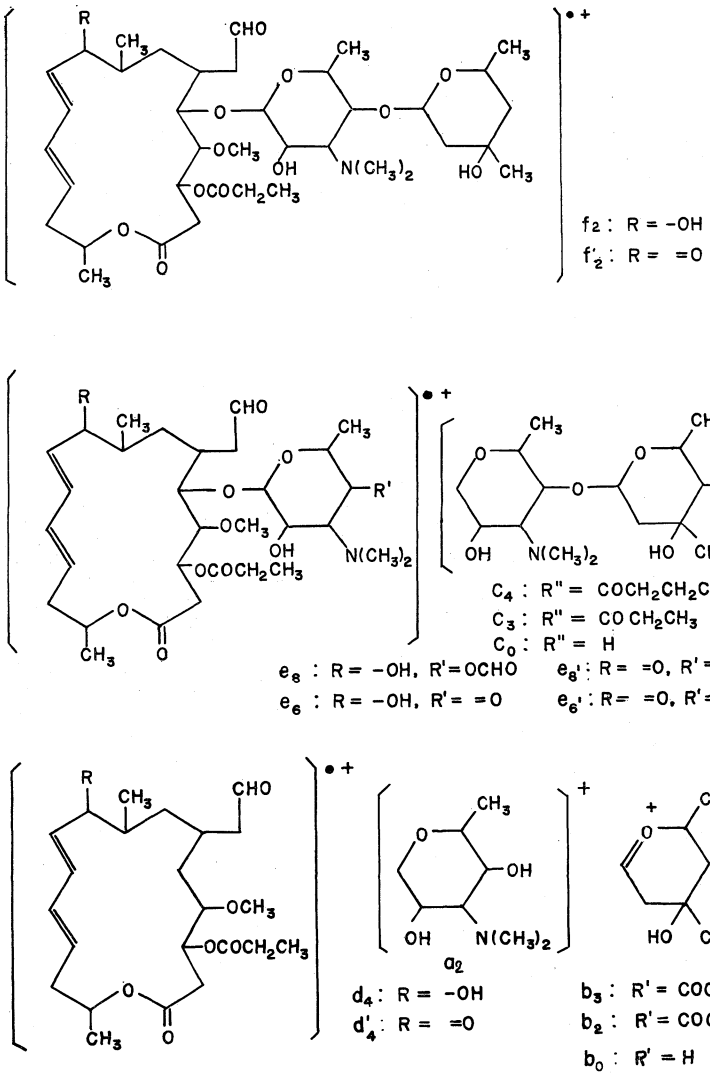


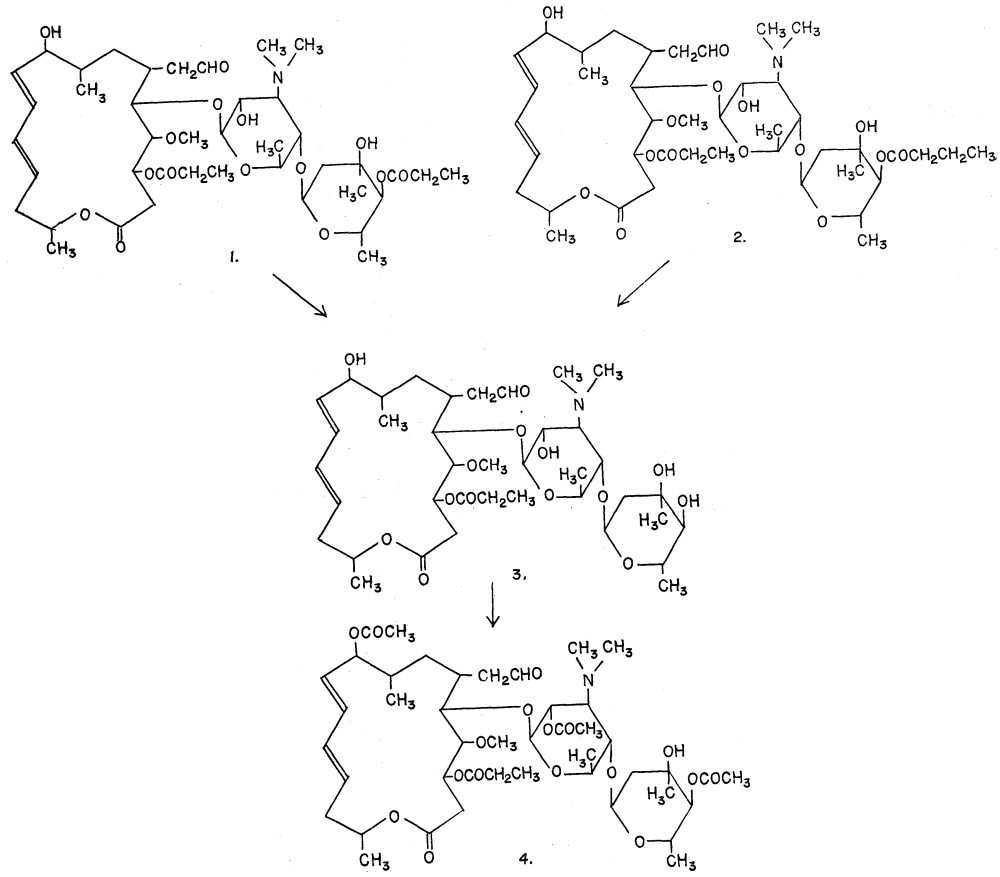
Chart 2. Structures of mass fragment ions.



Confirmation of this structure, particularly the stereochemistry, was required, since steric configuration is not always reflected in the mass spectrum.

Treatment of SF-837 A_2 with *Mucor spinescens*, as carried out for the deacylation

Chart 3. Correlation between SF-837 A_2 (**2**) and SF-837 (**1**).



of the leucomycin Fr group[2], gave de-*n*-butyryl SF-837 A_2 (**3**). The NMR spectrum of **3** showed an aldehyde proton signal at δ 9.69, olefinic proton signals at 5.5~6.8, a methoxyl proton signal at 3.57 and a di-methylamino proton signal at 2.57. The spectrum resembled more closely those of the leucomycin Ac group, which bear an O-acetyl group at C-3 of the lactone ring, than those of the leucomycin Fr group which have a free hydroxy group at this position[3]. The mass spectrum of this compound (Fig. 3) showed a molecular ion at m/e 757, a mycarose fragment ion $\mathbf{b}_0$ at m/e 145, a mycarosyl mycaminose ion $\mathbf{c}_0$ at m/e 318, and O-propionyl lactone fragment ions at

Fig. 3. Mass spectrum of 4''-de-*n*-butyryl SF-837 A_2 (**3**).

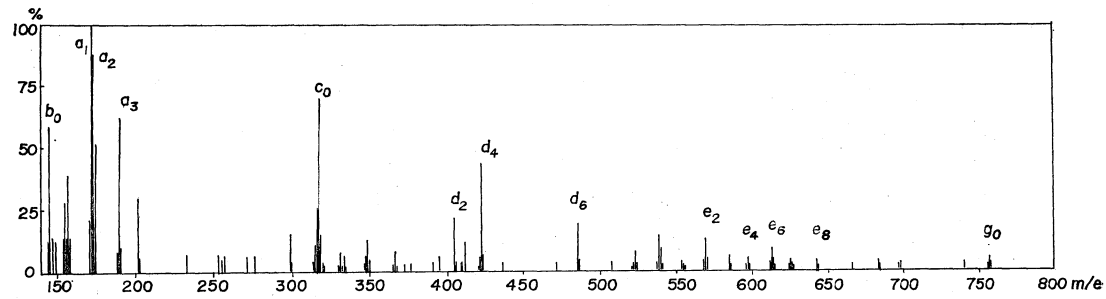
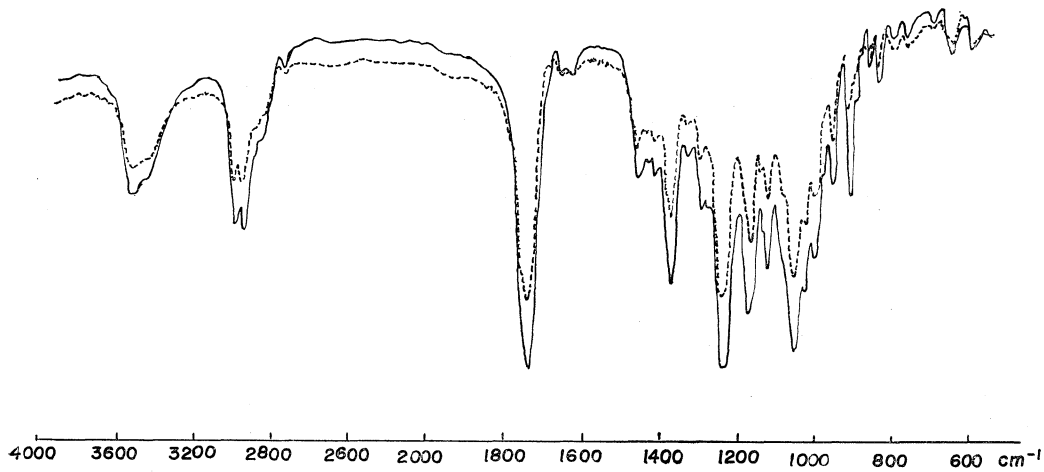


Fig. 4. IR spectra of triacetyl-4''-de-*n*-butyryl SF-837 A_2 (**4**) (——) and triacetyl-4''-de-propionyl SF-837 (-------) in KBr.



m/e 486 (d_6), 423 (d_4) and 405 (d_2). No peak arising from an O-*n*-butyryl mycarose moiety was detected. Accordingly, structure **3** was assigned to this compound.

Similar treatment of SF-837 gave depropionyl SF-837, indistinguishable from **3** by mass spectrum and TLC comparison. Acetylation of **3** with acetic anhydride in pyridine gave a crystalline tri-O-acetate (**4**), whose mass spectrum showed a strong deacetyloxy peak at m/e 824, indicating acetyl substitution at C-4'' of the mycarose moiety. Thus compound **4** may be regarded as the diacetyl derivative of a new macrolide, which possesses an acetyl group on the mycarose moiety, and retains a propionyl group on the lactone ring. Compound **4** was again identical in all respects with the tri-O-acetyl derivative of 4''-depropionyl SF-837 [identical m. p., $[\alpha]_D$, IR, NMR, mass and Rf value on TLC (Table 1)]. Fig. 4 illustrates the IR spectra of both compounds.

Table 1. Relative Rf values of various derivatives of SF-837, A_2, A_3 and A_4 on silica gel TLC developed with benzene-acetone (2 : 1)

Compound	Relative Rf*
Diacetyl SF-837 A_2	1.48
Triacetyl-4''-de-*n*-butyryl SF-837 A_2 (**4**)	1.37
Triacetyl-4''-depropionyl SF-837 (**4**)	1.37
Monoacetyl-dehydro SF-837 A_2 (**8**)	1.32
Monoacetyl SF-837 A_4 (**8**)	1.32
Monoacetyl-dehydro SF-837 (**7**)	1.26
Monoacetyl SF-837 A_3 (**7**)	1.26
Dehydro SF-837 A_2 (**6**)	1.13
SF-837 A_4 (**6**)	1.13
Dehydro SF-837 (**5**)	1.06
SF-837 A_3 (**5**)	1.06
SF-837 A_2 (**2**)	1.06
SF-837 (**1**)	1.00
4''-De-*n*-butyryl SF-837 A_2 (**3**)	0.48
4''-Depropionyl SF-837 (**3**)	0.48

* Relative Rf values were determined with an internal standard of SF-837 (**1**) assigned Rf value of 1.00.

Thus, antibiotic SF-837 A_2 with the exception of the acyl group on the mycarose moiety has the same structure and absolute configuration as SF-837.

Structure of SF-837 A_3

Antibiotic SF-837 A_3 exhibited a strong UV absorption band at 280 mμ, suggesting an α, β, γ, δ-unsaturated carbonyl chromophore. The IR bands at 1680, 1640 and 1600 cm^{-1} might be assignable to this chromophore. The NMR spectrum shown in

Fig. 5. NMR spectrum of antibiotic SF-837 A_3 (**5**) in $CDCl_3$.

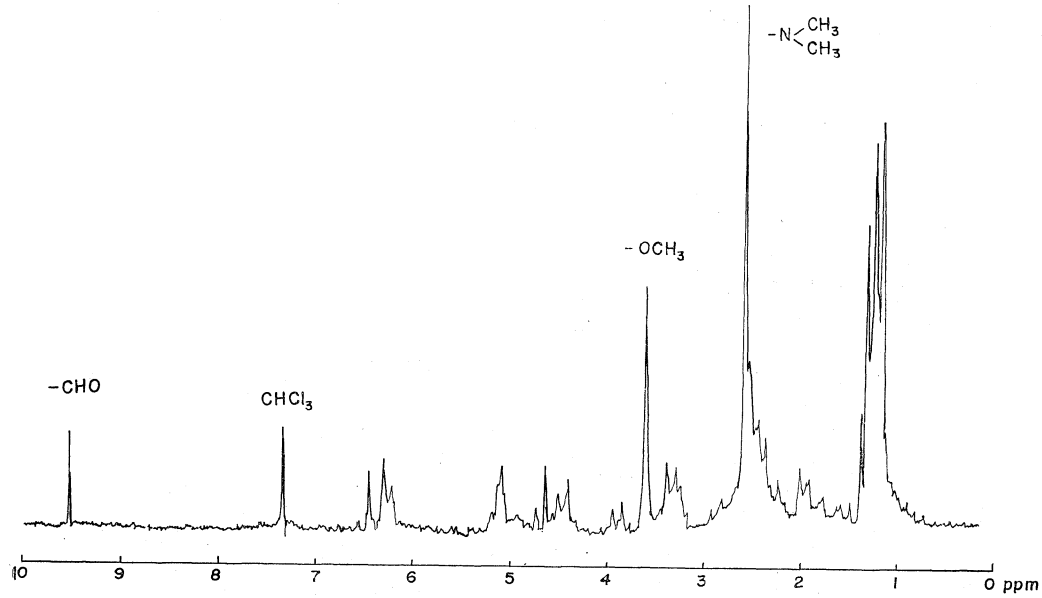


Fig. 6. Mass spectrum of antibiotic SF-837 A_3 (**5**).

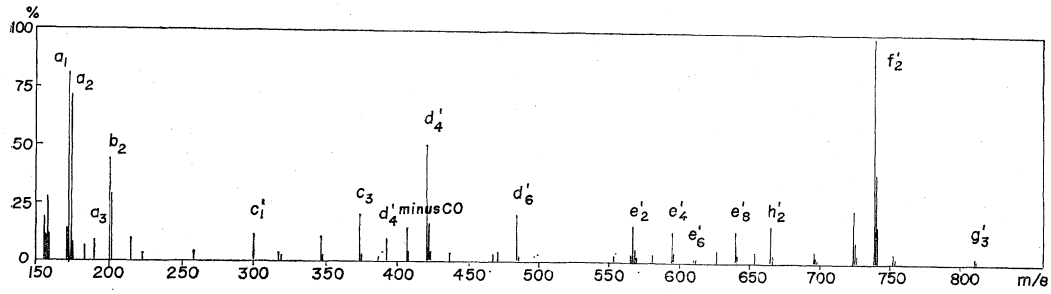


Fig. 5 was significantly different in the olefinic proton region from that of SF-837, reflecting an electronic change of the conjugated chromophore. Other signals, such as those of the aldehyde, anomeric, methoxyl and dimethylamino protons, which appear in the spectrum of SF-837 were all recognizable in the spectrum of SF-837 A_3.

It was mentioned in the previous paper[1] that the molecular formula of SF-837 A_3 ($C_{41}H_{65}NO_{15}$) is two hydrogens less than that of SF-837, and that SF-837 A_3 formed a monoacetate under conditions in which SF-837 formed a diacetate derivative. Both compounds gave two molecules of propionic acid on hydrolysis. These results in conjunction with the change in the chromophore as described above, led to the suggestion that SF-837 A_3 is the oxidation product of the allylic alcohol of SF-837. Indeed, the mass spectral fragmentation pattern of SF-837 A_3 strongly supported this structural proposal.

As shown in Fig. 6, sugar fragment ions arising from the mycaminose ($\mathbf{a}_1$, $\mathbf{a}_2$ and $\mathbf{a}_3$), mycarose ($\mathbf{b}_2$) and mycarosyl mycaminose ($\mathbf{c}_1$ and $\mathbf{c}_3$) moieties of SF-837 A_3 were a replica of those of SF-837. This indicates that SF-837 A_3 possesses a 4''-O-propionyl mycarosyl-mycaminose unit as a partial structure. Fragment ions involving the

Fig. 7. Mass spectrum of monoacetyl SF-837 A_3 (7).

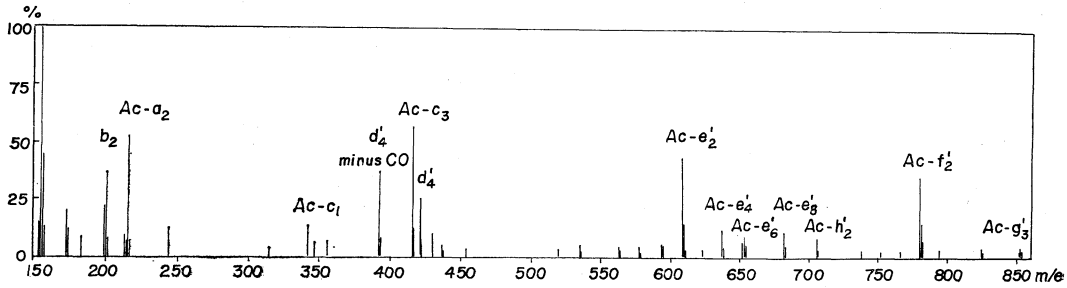
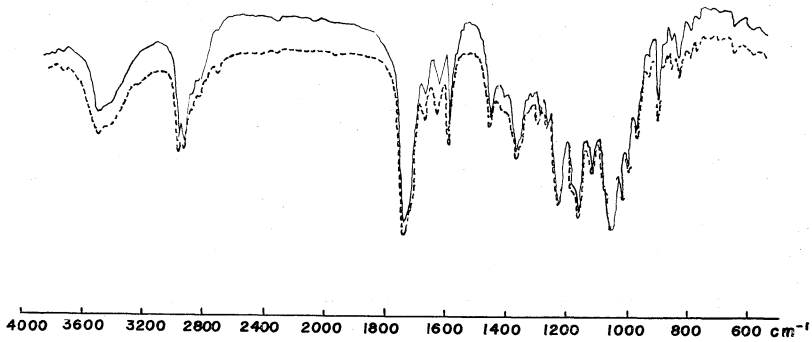


Fig. 8. IR spectra of monoacetyl dehydro SF-837 (7) (——) and monoacetyl SF-837 A_3 (-------) in KBr.



lactone ring, on the other hand, were at two mass units lower than those of SF-837. These fragment ions included lactone fragment peaks ($\mathbf{d}_4'$ and $\mathbf{d}_6'$), mycaminosyl lactone peaks ($\mathbf{e}_2'$, $\mathbf{e}_4'$, $\mathbf{e}_6'$ and $\mathbf{e}_8'$), a thermal peak ($\mathbf{h}_2'$), a depropionyloxy peak ($\mathbf{f}_2'$) and a molecular ion ($\mathbf{g}_3'$). Particularly interesting was the absence of a fragment ion $\mathbf{d}_2'$ ($\mathbf{d}_4'$ minus H_2O), and appearance of a decarbonyl ion ($\mathbf{d}_4'$ minus CO) of considerable intensity at m/e 393. This was rationalized by a replacement of an −OH group in the structure of SF-837 with a C=O group in the structure of SF-837 A_3.

Further evidence was obtained from the mass spectrum of a mono-O-acetyl derivative of SF-837 A_3 (Fig. 7). While fragment ions containing the mycaminose portion, such as Ac-**a**, Ac-**c**, Ac-**e**, Ac-**h**, Ac-**f** and Ac-**g** showed an increment of 42 mass units due to the new acetyl group at the C-2′ position, mass numbers of other fragment ions such as the lactone peaks ($\mathbf{d}_4'$ and $\mathbf{d}_4'$ minus CO) and a mycarose peak ($\mathbf{b}_2$) were unchanged.

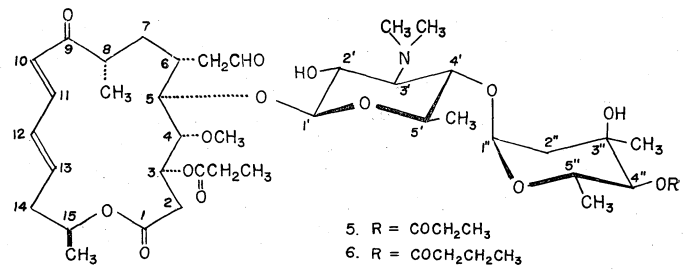
In order to chemically confirm the structure of SF-837 A_3, an attempt was made to convert SF-837 directly into SF-837 A_3 by oxidation with activated manganese dioxide, a procedure which has been used for the conversion of leucomycin A_3 into carbomycin B[4]. It was shown that the oxidation product (**5**) of SF-837 was indeed identical with SF-837 A_3 in respect to UV, NMR and mass spectra and Rf value on TLC (Table 1). Acetylation of **5** with acetic anhydride in pyridine at room temperature yielded a crystalline monoacetyl derivative (**7**), whose m.p., IR, NMR and mass spectra and Rf value on TLC were identical with those of monoacetyl SF-837 A_3. Fig. 8 shows the IR spectra of both compounds.

The successful correlation of antibiotic SF-837 A_3 with SF-837 not only confirmed the structure of SF-837 A_3, proposed from the physico-chemical investigations, but also established its absolute stereochemistry.

Structure of SF-837 A_4

Once the structure of antibiotic SF-837 A_3 was established, the structural determination of SF-837 A_4 followed analogously. The UV, IR and NMR spectra of SF-837 A_4 were essentially those of SF-837 A_3, suggesting the presence of the same chromophore in the two compounds. SF-837 A_4 contains one propionyl and one *n*-butyryl group, as does SF-837 A_2, and its molecular formula ($C_{42}H_{67}NO_{15}$) is two hydrogens less than that of SF-837 A_2. These data were sufficient to suggest that SF-837 A_4 is an oxidized form of SF-837 A_2 as shown in Chart 4.

Chart 4. Structures of antibiotic SF-837 A_3 (**5**) and A_4 (**6**).

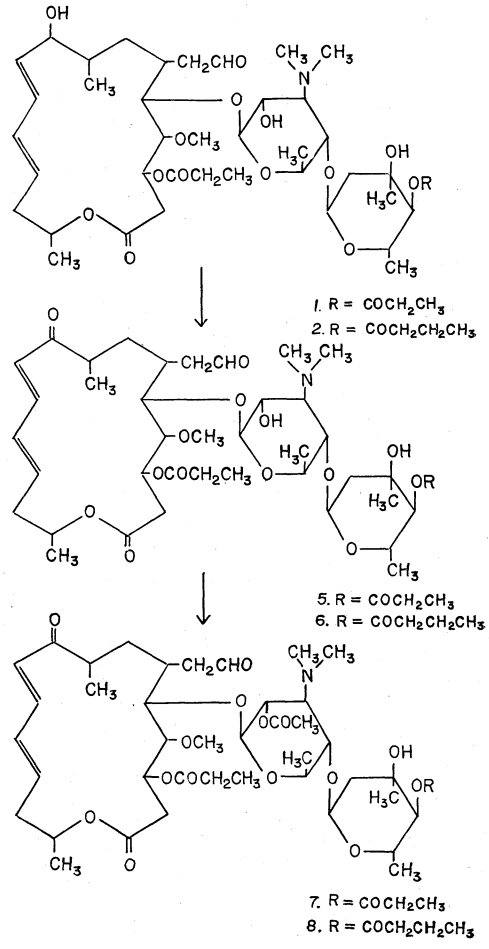


In agreement with the structure proposed, mass fragment ions of SF-837 A_4 and its monoacetate involving the macrocyclic lactone portion, were a replica of those of SF-837 A_3 and its monoacetate. Fragment ions containing the mycarose portion coincided with those of SF-837 A_2 and its diacetate. Therefore, the presence of a propionyl group on the dehydro lactone portion, and *n*-butyryl group on the mycarose portion was indicated.

Manganese dioxide oxidation of SF-837 A_2 gave the dehydro derivative (**6**), which was identical with SF-837 A_4, as shown by comparison of the physical properties of the free macrolides as well as those of the monoacetates. Thus, the structure of antibiotic SF-837 A_4 was determined by correlating it with SF-837 A_2, whose structure had been determined by correlation with SF-837.

To the best of our knowledge, simultaneous biological production of the re-

Chart 5. Conversion of antibiotic SF-837 (**1**) and A_2 (**2**) into SF-837 A_3 (**5**) and A_4 (**6**).



duced macrolides (SF-837 and SF-837 A_2) and their respective oxidized counterparts (SF-837 A_3 and A_4) involving such a conjugated system has not previously been reported. In this respect, it may be of interest to investigate the biosynthetic relationship of both types of macrolides.

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 with TMS as the internal standard. Chemical shifts are given on the δ scale. Mass spectra were taken with a JMS-01SG high resolution mass spectrometer at 75 eV, using an electric detector coupled with the direct inlet system.

TLC was performed with silica gel (Camag, Switzerland) or alumina (Merck, Germany) as the adsorbent. Indication was effected by spraying with 10 % sulfuric acid followed by heating with an ultrared lamp. Since Rf values of macrolides are greatly affected by (i) quantity of sample, (ii) water content of the adsorbent, and (iii) temperature of the atmosphere, relative Rf values were determined by comparison with the Rf of SF-837, which was run as an internal standard, partially overlapped with test compounds. Table 1 summarizes the Rf values of SF-837 A_2, A_3, A_4 and their derivatives, relative to the Rf of SF-837.

Preparation of 4''-de-*n*-butyryl SF-837 A_2 (**3**)

A suspension of *Mucor spinescens* IAM 6071 in a medium (500 ml) containing 2 % sucrose, 1 % glucose, 1 % soybean meal, 1 % peptone and 1 % Pharmamedia (pH 7) was shaken at 28°C for 70 hours. After addition of an aqueous solution of SF-837 A_2 (300 mg) (pH 4), the culture was shaken for further 40 hours. The broth filtrate and washings of the mycelia were combined and extracted with ethyl acetate (200 ml) at pH 8.5. The deacylated product in the ethyl acetate layer was transferred into aqueous acid (100 ml) at pH 3.0, and transferred again into ethyl acetate (75 ml) at pH 8.5. The final ethyl acetate layer was dried over sodium sulfate, and evaporated to yield a pale yellow powder (230 mg). This was dissolved in benzene (10 ml), and chromatographed on a column of silica gel (20 ml), developed with benzene - acetone (1 : 1). Effluents containing **3** were collected, and concentrated to dryness to give 4''-de-*n*-butyryl SF-837 A_2 (**3**) as a white powder (160 mg). m.p. 122~128°C, $[\alpha]_D^{27}$ −53° (*c* 1, in ethanol).

Anal. Calcd. for $C_{38}H_{63}NO_{14}$ (MW 757): C 60.22, H 8.38, N 1.85 %
Found: C 60.28, H 7.96, N 1.69 %

On silica gel TLC developed with benzene - acetone (4 : 1), **3** gave a spot which was completely superimposable on a spot of 4''-depropionyl SF-837.

2',4'',9-Tri-O-acetyl-4''-de-*n*-butyryl SF-837 A_2 (**4**)

A solution of **3** (90 mg) and acetic anhydride (0.5 ml) in pyridine (2 ml) was kept at 3°C for 22 hours, and then poured into ice-water. A crude product precipitated was collected by filtration and dried *in vacuo*. Crystallization from carbon tetrachloride (1 ml) gave colorless needles of 2',4'',9-tri-O-acetyl-4''-de-*n*-butyryl SF-837 A_2 (**4**) (62 mg). m. p. 123~126°C alone or admixed with tri-O-acetyl-depropionyl SF-837, $[\alpha]_D^{27}$ −68° (*c* 1, in ethanol).

Anal. Calcd. for $C_{44}H_{69}NO_{17}$ (MW 883): C 59.78, H 7.86, N 1.58 %
Found: C 60.12, H 7.53, N 1.49 %

Mass fragment ions: m/e 883 (M^+), 856 (M^+ minus CO), 824 (deacetyloxy), 465 (acetyl-propionyl deoxylactone), 216 (acetyl mycaminose), and 187 (acetyl mycarose). The IR

(Fig. 4), NMR and mass spectra were identical with those of tri-O-acetyl depropionyl SF-837.

Preparation of 4''-depropionyl SF-837 and its triacetate

A crude deacylated product (380 mg) was prepared from SF-837 (500 mg) by the procedures similar to those used for SF-837 A_2. Chromatographic purification of this product over silica gel gave as a white powder, 4''-depropionyl SF-837, 280 mg. m.p. 122~124°C, $[\alpha]_D^{28}$ −52° (*c* 1, in ethanol).

Anal. Found: C 60.92, H 8.23, N 1.72 %

Acetylation of 4''-depropionyl SF-837 (200 mg) with acetic anhydride (0.8 ml) and pyridine (3 ml), followed by crystallization from carbon tetrachloride (2 ml) yielded colorless needles of 2',4'',9-tri-O-acetyl-4''-depropionyl SF-837 (150 mg), m.p. 124~127°C, $[\alpha]_D^{28}$ −70° (*c* 1, in ethanol).

Anal. Found: C 60.23, H 7.63, N 1.52 %

Conversion of SF-837 (**1**) into SF-837 A_3 (**5**) by manganese dioxide oxidation

To a solution of **1** (400 mg) in acetone (20 ml) was added activated manganese dioxide (3.5 g) previously dried at 120°C for 2 hours, and the mixture was stirred at 28°C for 30 hours. Insoluble materials were removed by filtration, and the filtrate was evaporated to a solid (370 mg). This was dissolved in benzene, and chromatographed on a column of silica gel (60 ml) developed with benzene-acetone (4 : 1). Effluents containing **5** were combined and evaporated to dryness to yield a white powder of a dehydro derivative (**5**). 110 mg. m.p. 121~124°C, $[\alpha]_D^{24}$ −43° (*c* 1, in ethanol). It showed a UV maximum at 280 mμ ($E_{1cm}^{1\%}$ 302).

Anal. Calcd. for $C_{41}H_{65}NO_{15}$ (MW 811): C 60.67, H 8.01, N 1.73 %
Found: C 60.38, H 7.82, N 1.69 %

The UV, NMR and mass spectra were in good agreement with those of SF-837 A_3.

Acetylation of **5** (60 mg) with acetic anhydride (0.3 ml) and pyridine (1 ml) at 5°C for 20 hours followed by crystallization from carbon tetrachloride gave the monoacetyl derivative (**7**) as colorless plates, 40 mg. m.p. 183~185°C alone or admixed with a sample of SF-837 A_3.

Anal. Calcd. for $C_{43}H_{67}NO_{16}$ (MW 853): C 60.48, H 7.91, N 1.64 %
Found: C 60.28, H 7.38, N 1.59 %

The IR (Fig. 8), NMR and mass spectra were identical with those of monoacetyl SF-837 A_3.

Conversion of SF-837 A_2 (**2**) into SF-837 A_4 (**6**) by oxidation

SF-837 A_2 (**2**) (300 mg) was oxidized in acetone with activated manganese dioxide (3 g), and dehydro SF-837 A_2 (**6**) was isolated by the procedures similar to those described above. Yield, 90 mg, m.p. 120~122°C, $[\alpha]_D^{24}$ −41° (*c* 1, in ethanol). It showed a UV band at 280 mμ ($E_{1cm}^{1\%}$ 295) in ethanol.

Anal. Calcd. for $C_{42}H_{67}NO_{15}$ (MW 825): C 61.09, H 8.12, N 1.70 %
Found: C 60.82, H 7.86, N 1.62 %

Mass fragment ions: m/e 825 (M^+), 738 (de-*n*-butyryloxy), 484, 421, 393 (macrocyclic lactone), 388 (*n*-butyryl mycarosyl mycaminose), 215 (*n*-butyryl mycarose), 190, 174, and 173 (mycaminose). The UV, NMR and mass spectra were in agreement with those of SF-837 A_4.

Acetylation of **6** (60 mg) with acetic anhydride (0.3 ml) in pyridine (1 ml), and crystallization from carbon tetrachloride (0.7 ml) gave the monoacetyl derivative (**8**) (40 mg). m.p. 165~168°C alone or admixed with an authentic sample of monoacetyl SF-837 A_4.

Anal. Calcd. for $C_{43}H_{69}NO_{16}$ (MW 867): C 60.33, H 8.12, N 1.63 %
Found: C 60.28, H 7.29, N 1.59 %

Mass fragment ions: m/e 867 (M^+), 839 (M^+ minus CO), 780 (de-*n*-butyryloxy). The IR, NMR and mass spectra were indistinguishable from those of monoacetyl SF-837 A_4.

Acknowledgement

The authors express their gratitude to Mr. KODAMA for mass spectra, Mr. KASHIWAKURA for NMR spectra and Miss SEKIYA for elemental analysis.

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