YS-822A, A NEW POLYENE MACROLIDE ANTIBIOTIC

II. PLANAR STRUCTURE OF YS-822A

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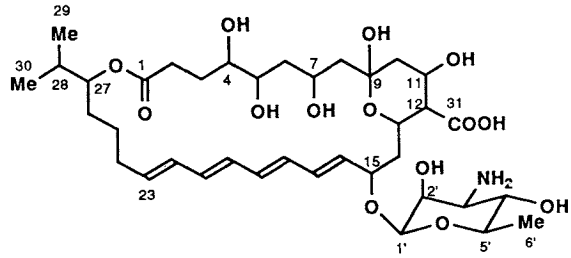
The planar structure of a new polyene macrolide antibiotic, YS-822A, which was isolated from the culture filtrate of a mutant strain H-8 of *Streptoverticillium eurocidicum* var. *asterocidicus* S-822, was established as **I** on the basis of spectroscopic evidences and by comparison of spectroscopic data with nystatin A_1 (**II**) and amphotericin A (**III**), representative polyene macrolide antibiotics. Molecular formula of YS-822A was established as $C_{37}H_{59}NO_{14}$ (MW 741) by elemental analysis, NMR, and FAB mass spectra. The UV spectrum of YS-822A was very similar to that of nystatin A_1, suggesting that YS-822A also has a conjugated all-*trans*-tetraene moiety. ^1H and ^{13}C NMR spectra of YS-822A showed a number of broad and overlapped signals, but the ^1H-^1H and ^{13}C-^1H COSY spectra implied the existence of a mycosamine moiety and several other partial structures. The connectivity of these partial structures was established by extensive 2D NMR experiments, including homonuclear Hartmann-Hahn and heteronuclear multiple-bond connectivity measurements, which led to the determination of the gross planar structure of YS-822A as **I**.

In the preceding paper[1], we described the production, isolation, characterization, and biological properties of a new polyene macrolide antibiotic, YS-822A. In this paper we wish to report our experimental results leading to planar structural elucidation of **I** (see Fig. 1).

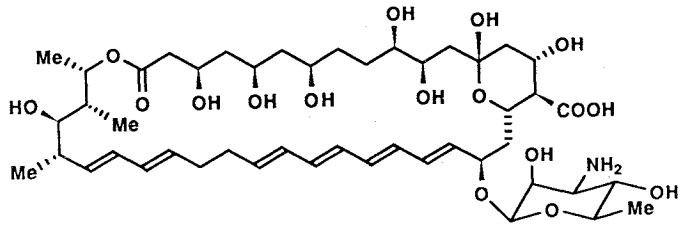
Results

The UV spectrum of YS-822A in methanol showed characteristic polyene-type absorptions very similar to that of nystatin A_1 (**II**)[2~4] with maxima at 290, 303, and 318 nm corresponding to an all-*trans*-tetraene antibiotic. A positive FAB mass spectrum (matrix: 3-nitrobenzyl alcohol) showed an ion at *m/z* 764 (nominal mass), accompanied with a less intense ion at *m/z* 742, which indicated that the former ion was assignable to MNa^+ and the latter to MH^+. These assumptions were confirmed by a shift of 16 mass unit from *m/z* 764 (MNa^+) to *m/z* 780 (MK^+) when a drop of aqueous potassium chloride solution was added to the FAB target. Further evidence of the MW of YS-822A to be 741 was obtained by the negative FAB

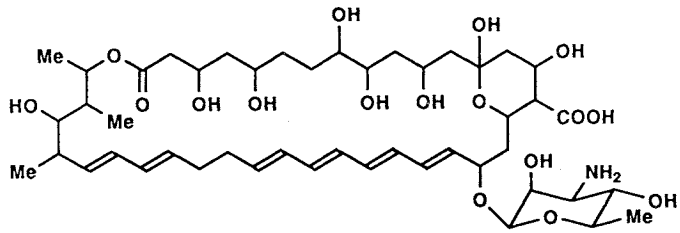
Fig. 1. Structures of YS-822A (**I**), nystatin A_1 (**II**), and amphotericin A (**III**).



YS-822A (**I**)



Nystatin A_1 (**II**)



Amphotericin A (**III**)

Table 1. ^{13}C and ^{1}H NMR data for YS-882A (**I**).

Carbon	^{13}C	^{1}H	Carbon	^{13}C	^{1}H
1	173.2 s		20	131.8 d	6.28
2	30.7 t	2.38, 2.52	21	131.5 d	6.15
3	28.1 t	1.65, 1.75	22	130.9 d	5.95
4	72.2 d	3.20	23	133.4 d	5.73
5	72.7 d	3.55	24	29.8 t	1.98, 2.10
6	38.9 t	1.42, 1.58	25	24.4 t	1.20, 1.48
7	67.4 d	4.31	26	29.8 t	1.45, 1.55
8	45.8 t	1.55, 1.61	27	76.0 d	4.78
9	97.0 s		28	31.4 d	1.75
10	44.3 t	1.10, 1.85	29	17.7 q	0.84 (3H, d, J=7 Hz)
11	65.6 d	4.00	30	18.5 q	0.86 (3H, d, J=7 Hz)
12	58.8 d	1.82	31	177.5 s	
13	65.2 d	4.20	1′	95.4 d	4.55
14	36.1 t	1.45, 2.18	2′	67.8 d	3.80
15	74.0 d	4.40	3′	56.2 d	2.92
16	136.2 d	6.05	4′	69.6 d	3.22
17	128.3 d	6.12	5′	72.4 d	3.32
18	132.9 d	6.35	6′	17.9 q	1.17 (3H, d, J=6 Hz)
19	131.2 d	6.20			

mass spectrum (matrix: triethanolamine) of the sample, which showed $(M-H)^-$ at m/z 740. From these results together with elemental analysis and ^1H, ^{13}C NMR data (see Table 1), the molecular formula of YS-822A was established as $C_{37}H_{59}NO_{14}$.

Although 1D ^1H and ^{13}C NMR spectra of YS-822A in DMSO-d_6 showed a number of broad and overlapped signals, ^{13}C-^1H COSY spectrum afforded the assignment of directly bonded carbons and protons (see Table 1), and ^1H-^1H COSY (see Fig. 2) spectrum implied the existence of several partial structures (**A**, **B**, **C**, and **D** as shown in Fig. 3). That is, the presence of mycosamine moiety (a partial structure **A**) was deduced from ^1H-^1H COSY correlation peaks (1'-H (δ 4.55)/2'-H (δ 3.80), 2'-H/3'-H (δ 2.92), 3'-H/4'-H (δ 3.22), 4'-H/5'-H (δ 3.32), and 5'-H/6'-H_3 (δ 1.17)), and comparison of its ^1H and ^{13}C chemical shifts with those in literature[5]. One side of the all-*trans*-tetraene moiety, the presence of which was predicted from the UV spectrum *vide supra* and was confirmed by these NMR spectra, was attached with an methylene (δ_C 29.8 t, δ_H 1.98 and 2.10), which was, in turn, connected with an methylene (δ_C 24.4 t, δ_H 1.20 and 1.48). The other side of the tetraene moiety was connected with a methine (δ_C 74.0 d, δ_H 4.40; adjacent to an oxygen), and it was probable from the ^1H-^1H COSY spectrum that this methine was connected with a unit consisted of two methylenes and three methines to compose a partial structure **B**. The partial structures **C** and **D** were also deduced from ^1H-^1H COSY correlations, even though there were several severely overlapped signals, for example there were 11 proton signals between δ_H 1.4 and 1.8. The ambiguity and the poor reliability to the assignments and proposed partial structures **C** and **D** were dissolved by homonuclear Hartmann-Hahn (HOHAHA) and heteronuclear multiple-bond connectivity

Fig. 2. ^1H-^1H COSY spectrum of YS-822A in DMSO-d_6 (300 K).

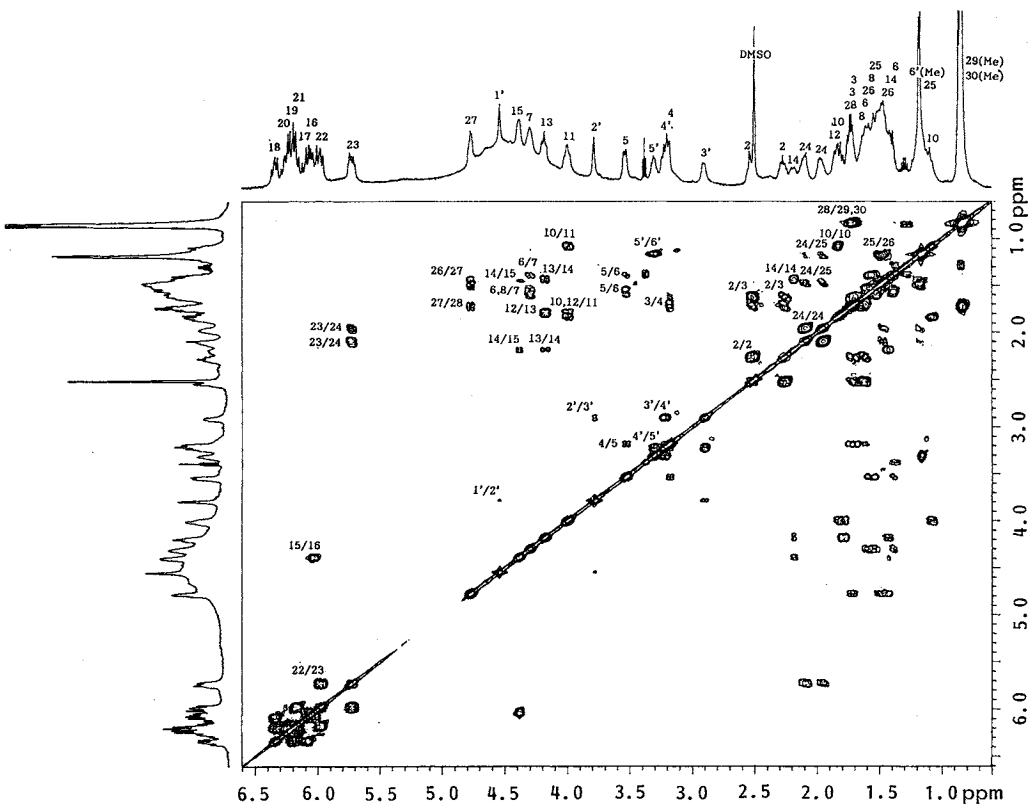
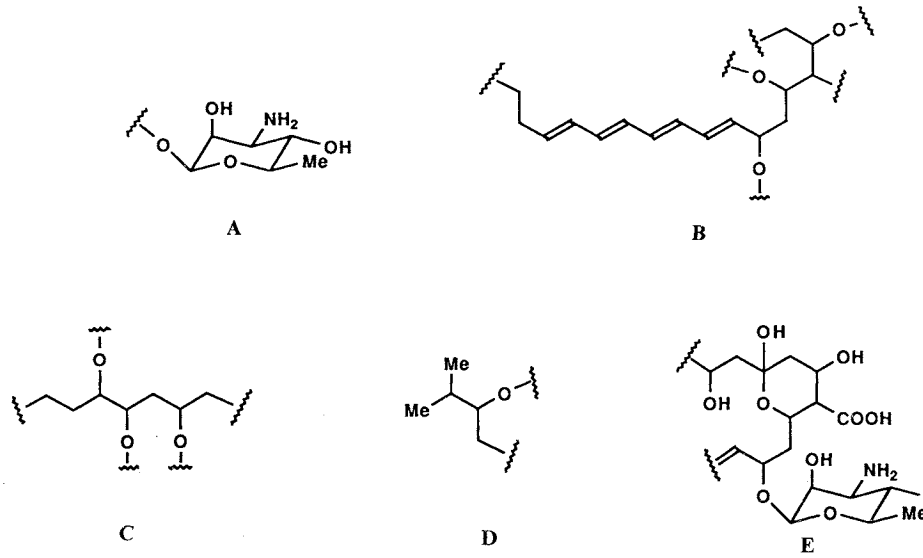


Fig. 3. Partial structures of YS-822A.



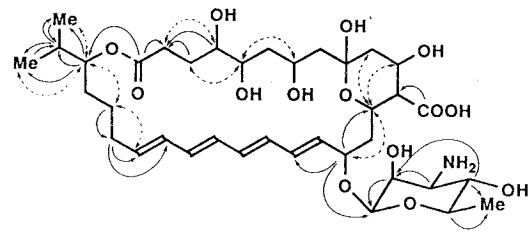
(HMBC) spectra *vide infra*.

A comparison of ^{1}H and ^{13}C chemical shifts between amphotericin A (**III**) in the literature[5] and YS-822A showed the presence of the same partial structure **E**. This structure **E** clarified not only the functional groups in the partial structure **B** but also connections between **A** and **B** and between **B** and **C**.

HOHAHA[6,7] and HMBC[8] measurements led not only to confirm the deductions *vide supra* but also to connect all the remaining fragments and quaternary carbons (see Fig. 4). That is, the distinct correlation peaks of a carbonyl carbon (C-1; δ 173.2) with 2-H (δ 2.38 and 2.52), and 27-H (δ 4.78) appeared on the HMBC spectrum, which established the connection between partial structures **C** and **D** through an ester group. On HOHAHA spectrum, a methine proton at δ 4.78 (27-H) showed correlation peaks with protons at δ 1.98 and 2.10 (24-H) through 1.45 and 1.55 (26-H), and 1.20 and 1.48 (25-H), and two methyl protons at δ 0.84 and 0.86 through 1.75 (28-H), which not only confirmed the partial structure **D** but also established the connection between **D** and **B**. Correlation peaks between C-15 (δ 74.0) and 1'-H (δ 4.55) and between C-31 (δ 177.5) and 12-H (δ 1.82) on HMBC spectrum supported the partial structure **E**.

Fig. 4. Results of HMBC and ^{1}H-^{1}H HOHAHA measurements of YS-822A in DMSO-d_6 (300 K).

Solid arrows denote correlation peaks between carbons (tail) and protons (head) in HMBC spectrum. Dotted lines indicate ^{1}H-^{1}H HOHAHA correlations after removal of ^{1}H-^{1}H-COSY ones.



YS-822A had nine degrees of unsaturation, all of which have already been assigned to four double bonds, two carbonyls (a lactone and a carboxylic acid), and three rings. So, all the oxygen functional groups at **C** should be hydroxyls.

Thus, the planar structure of YS-822A was determined as **I**.

Discussion

YS-822A (**I**) was established as a new antifungal macrolide with a tetraene and a mycosamine moieties,

though there are many known antibiotics with the similar functional groups[2~5,9], for example, nystatin, amphotericins, tetrins, and tetramycin.

2D NMR spectroscopy showed a very strong power for the planar structure determination of YS-822A, although there were a number of broad and overlapped signals on 1D ^{1}H and ^{13}C NMR spectra.

On the mass spectroscopic experiments, a positive FAB collision-activated dissociation (CAD) spectrum was also taken by B/E-constant linked scanning mode. When MNa^+ peak was chosen as the precursor ion, *m/z* 764.4 (MNa^+) showed daughter ions at *m/z* 746 $(MNa-H_2O)^+$, *m/z* 601 $(MNa-163)^+$, *m/z* 583 $(MNa-(163+H_2O))^+$, and *m/z* 186 $(163+Na)^+$, where 163 corresponds to the MW of mycosamine. Characteristic fragment ions at *m/z* 702, 557, and 539 represent 44 mass unit loss from *m/z* 746, 601, and 583, respectively, which may be produced by $-CO_2-$ (charge-mediated fragmentation) and/or the terminal C_3H_8 loss (charge-remote fragmentation)[10].

Experimental

General Analytical Methods

MP was measured on a Mel-temp capillary melting point apparatus (Laboratory Devices) and uncorrected. Optical rotation was determined on a Jasco DIT-140. UV spectra were measured on a Hitachi U-3200 spectrophotometer. An IR spectrum was recorded on a Nicolet model 205 FT-IR spectrometer. FAB-MS were obtained with a Jeol HX-110 mass spectrometer equipped with a 6 KeV Xe FAB gun at 10 KV ion acceleration voltage. The daughter spectrum was measured in the B/E-constant linked scanning mode applying collision activation using He gas. NMR spectra were measured on a Bruker AM-500 NMR spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C nuclei) at 300 K using DMSO-d_6 as solvent.

YS-822A (I)

MP 265°C; $[\alpha]_D^{23}$ +21° (*c* 0.1, pyridine); UV λ_{max}^{MeOH} nm (ε) 290 (27,400), 303 (39,900), 318 (36,100); IR (KBr) cm^{-1} 3400, 1710, 1570, 1060; (+)FAB-MS (matrix: 3-nitrobenzyl alcohol): *m/z* 764 (MNa^+; intense peak), 742 (MH^+), and 724 $(MH-H_2O)^+$; (+)FAB-MS (matrix: 3-nitrobenzyl alcohol on addition of potassium chloride): *m/z* 780 (MK^+), and 764 (MNa^+); (−)FAB-MS (matrix: triethanolamine): *m/z* 740 ($(M-H)^-$); Calcd for $C_{37}H_{59}NO_{14}$: C 59.90, H 8.02, N 1.89. Found: C 53.26, H 7.38, N 2.02. ^{1}H and ^{13}C NMR in Table 1. ^{1}H-^{1}H COSY spectrum in Fig. 2. Results of HMBC and ^{1}H-^{1}H HOHAHA experiments in Fig. 4.

Acknowledgments

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