PHYSICO-CHEMICAL AND MICROBIOLOGICAL COMPARISON OF NYSTATIN, AMPHOTERICIN A AND AMPHOTERICIN B, AND STRUCTURE OF AMPHOTERICIN A

A. ASZALOS*

Division of Drug Biology, Food and Drug Administration,
Washington, D.C., 20204, U.S.A.

A. BAX

National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases,
National Institutes of Health,
Bethesda, Maryland 20205, U.S.A.

N. BURLINSON

Department of Chemistry, University of British Columbia,
Vancouver, British Columbia VGT 1Y6, Canada

P. ROLLER

National Cancer Institute, National Institutes of Health,
Bethesda, Maryland 20205, U.S.A.

C. MCNEAL

Department of Chemistry, Texas A&M University,
College Station, Texas 77843, U.S.A.

(Received for publication April 13, 1985)

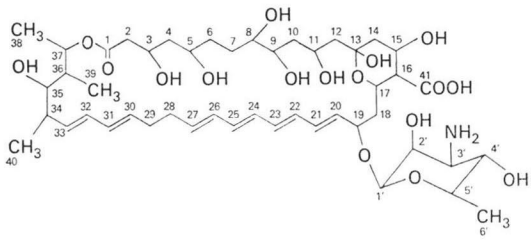
Two polyene antibiotics, nystatin and amphotericin A, were compared by physico-chemical and microbiological methods. The two antibiotics were found to have the same molecular weight, 926, by plasma desorption and electron-impact MS. However, ^{13}C NMR spectrometry and HPLC studies indicated that the two molecules are different. The 200 MHz NMR studies indicated a chemical environment of 24 carbons of amphotericin A identical with that of the carbons of amphotericin B and nystatin.

The structure of amphotericin A is identical with that of amphotericin B, except that there is a single bond between carbons 28 and 29 instead of a double bond, as shown by two-dimensional NMR studies.

Amphotericin A (AA), a tetraenic antibiotic, was discovered[1] in the fermentation broth of the heptaenic antibiotic amphotericin B (AB). While the structure of AB was established earlier[2], no rigorous structural studies were reported on AA, except that the sugar moiety in this antibiotic was also found to be mycosamine[3], as in AB. For some time, AA was thought to be identical to another tetraenic antibiotic, nystatin (NY), whose structure was also reported[4]. Later reports indicated different chromatographic mobility[5] and distribution in countercurrent systems[6] of AA and NY. However, the purity of the samples used in these early studies was not well defined.

We wished to compare some microbiological and physico-chemical characteristics of more purified NY and AA preparations, drug standards, since both of these polyenic antibiotics occur in commercial drug preparations[7]. In the present paper, we describe some of these comparative studies, and show

Fig. 1. Structure of AA.



by two-dimensional (2D) NMR studies a proposed structure for AA as shown in Fig. 1.

Materials and Methods

Antibiotics and Reagents

Purified NY (USP Ref Std: K, potency 5,900 U/mg), purified AB (USP Ref Std: B-2, potency 986 U/mg) and purified AA (USP Ref Std: HV-718) were used. Solvents and chemicals were spectrograde or commercially available grades and were used without further purification.

N-Acetyl Nystatin: *N*-Acetyl NY was prepared by mixing 1 g of NY into a mixture of 25 ml of MeOH and 2 ml of Ac_2O at room temp. After the NY dissolved, the methanolic soln was kept at room temp in the dark for an additional 18 hours. Then 300 ml of ether was added, followed by 600 ml of petroleum ether. The precipitate that formed was centrifuged, washed with petroleum ether in a centrifuge tube and dried. This material was then dissolved in 20 ml of MeOH and reprecipitated with ether and petroleum ether, washed with petroleum ether and dried. The yield was 700 mg. The material obtained, *N*-acetyl NY, appeared as one spot in all of the TLC systems described below. Nonaqueous titration indicated NY acid 1,002 and NY base 0. The *N*-acetyl number determination, 4.2%, indicated one acetyl function per 1,000 equivalent weight. IR spectra indicated the presence of an additional amide group (1623 cm^{-1}) compared to that of the parent compound.

N-Acetyl Amphotericin A: *N*-Acetyl AA was prepared as described for *N*-acetyl NY. All analytical values were the same as for *N*-acetyl NY.

N-Acetyl Amphotericin B: *N*-Acetyl AB was prepared essentially as *N*-acetyl NY, except that 1 g of AB was dissolved in 300 ml of MeOH and 35 ml of Ac_2O. All analytical values were the same as for *N*-acetyl NY.

Electron Impact Mass Spectrometry (EI-MS)

N-Acetyl NY and *N*-acetyl AA were pertrimethylsilylated, using bistrimethylsilyl trifluoroacetamide reagent, in a capillary tube. The pertrimethylsilylated samples were placed directly into a Jeol model JMS OISG-2 double focusing mass spectrometer. The ionization potential was 70 eV, trap current 200 μA and accelerating voltage 5 kV. Samples were introduced in the capillary tube and spectra were taken at 250~300°C. High resolution data were collected on Ilford Q-2 photoplates at approx 20,000 resolution. Plates were read in a Jeol model JMA-1C-0 data system. Perfluorokerosene was used as a standard.

Plasma Desorption Mass Spectrometry (PD-MS)

PD-MS of NY, AA and AB was carried out using the instrument and method described by MACFARLANE and TORGERSON[8]. Briefly, the production of molecular ions was based on the bombardment of the sample with high energy ^{252}Cf fission fragments. Samples were electro-deposited, using RbI with a soln of the polyene antibiotics. The masses of individual ions were calculated by the time-of-flight method.

High Performance Liquid Chromatography (HPLC)

HPLC was performed by using a modular apparatus equipped with a constant flow pump, a valve-type injector (model 6000 solvent delivery system, Waters Associates, Milford, MA) and a fixed wavelength (313 nm) UV detector (model 440, Waters Associates). A stainless steel column (4.6× 250 mm) was packed with fully porous 10 μm silica particles to which a mono-molecular layer of octadecylsilane was chemically bonded (Bondapak C_{18}, Waters Associates). Columns were washed with MeOH - H_2O each day after use. NY and AA standards were dissolved in DMSO to 10 mg/ml and diluted 1: 20 with MeOH before use. Flow rate varied between 0.4 and 2.0 ml/minute, depending on column pressure.

NMR Spectrometry

^{13}C NMR spectrometry of the underivatized NY and AA as well as the *N*-acetyl derivatives of NY, AA and AB was carried out at 5~8°C. The solvent was 10% D_2O in pyridine-d_5. A Varian XL-200 superconducting FT-NMR spectrometer was used with the following parameters: Spectrum acquisition, 50.3 MHz; spectral width, 15,083 Hz (300 ppm); acquisition time, 0.5 second; pulse delay, 0.5 second; filter bandwidth, ±8,300 Hz; transmitter pulse, 5 seconds (approx 30° flip angle). The Fourier transformation was done by using floating point mathematics. The insensitive nuclear enhanced polarization transfer (INEPT) spectrum was obtained by a pulse sequence as described by BURUM and ERNST[9].

2D NMR experiments were carried out on a Nicolet 500 MHz spectrometer at 60°C. The amounts of sample used were 10 mg in 0.5 ml of DMSO-d_6 in a 5-mm sample tube for ^1H experiments and 50 mg in 2 ml of DMSO-d_6 in a 10-mm sample tube for ^{13}C experiments. The ^1H-^1H shift correlation spectra were obtained by means of the correlated spectroscopy technique[10~14], using sine bell[15] digital filtering in both dimensions. Acquisition times in both t_1 and t_2 dimensions were 154 milliseconds for measurements on AB and 174 milliseconds for measurements on AA. A data matrix size of 512×1,024 was employed for both compounds. Spectra were displayed in the absolute value mode. Total measuring time for each of the two spectra was approx 4 hours.

The ^{13}C-^1H chemical shift correlation spectra were recorded in the phase-sensitive mode for improved resolution and sensitivity. To record the phase-sensitive spectra, a modified version[16] of the standard heteronuclear shift correlation experiment[14,17~20] was used. Total data accumulation time for each of the two 2D spectra was 10 hours. A Lorentzian to Gaussian transformation digital filter[21] was used in the ^{13}C dimension to improve spectral resolution.

Microbiological Assay

For the microbiological comparison of NY and AA, three sensitive organisms were used: *Candida albicans* (ATCC e10231), *C. tropicalis* (ATCC 13803) and *Saccharomyces cerevisiae* (ATCC 2601). The standard tube dilution assay was performed as described in the Code of Federal Regulations[22].

Results and Discussion

Mass Spectrometry

Ions observed in the EI-MS of pertrimethylsilylated *N*-acetyl NY and pertrimethylsilylated *N*-acetyl AA are given in Table 1. Fragmentation patterns of both compounds are similar up to *m/z* 404. At this *m/z*, a strong ion appears in the spectra of both compounds, which is assignable to the

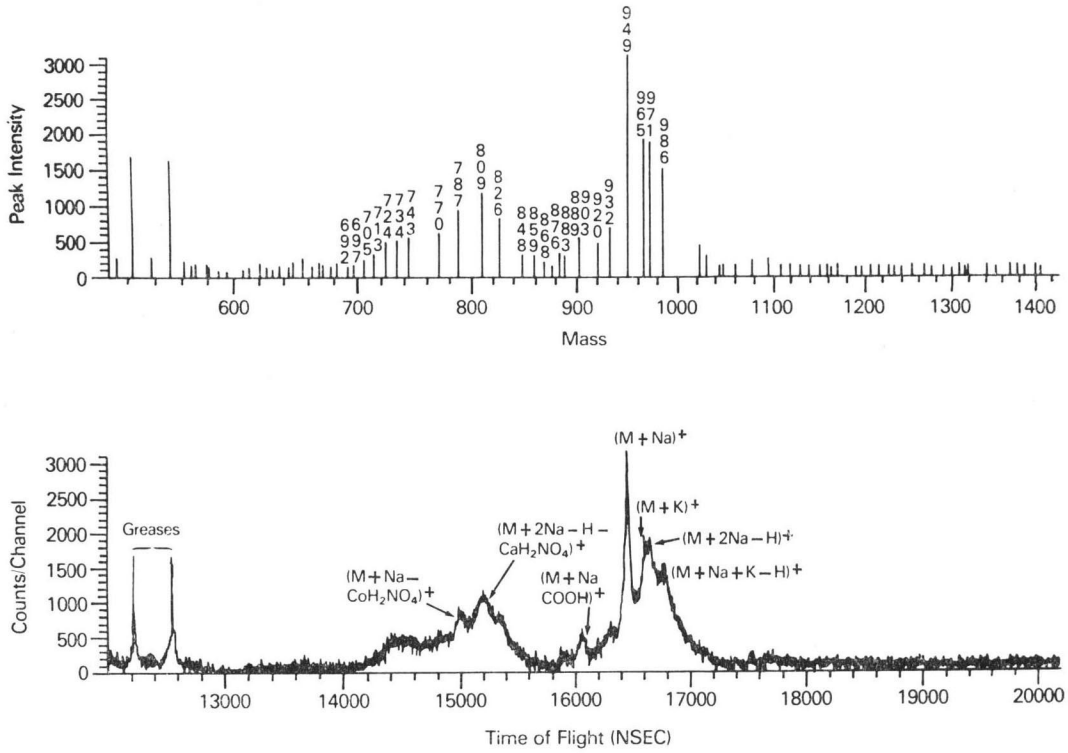
Table 1. Comparison of ions obtained by EI-MS from pertrimethylsilylated *N*-acetyl NY and pertrimethylsilylated *N*-acetyl AA.

m/z[a]		*m/z*	
NY	AA	NY	AA
243 s	243 w	883 w	883 s
244 w	244 s	—	959 s
259	259	1,463 s	—
273	273	1,480 s	—
332	332	1,553 s	—
343 m	344 m	—	1,557
348 s	348 m	1,574 w	—
390	390	—	1,685 s
404 s[b]	404 s[b]	1,725 w	—
—	792	1,759 w (M^+)	1,759 w (M^+)

[a] s: Strong, w: weak, m: medium.
[b] High resolution.

Fig. 2. Positive and negative ion plasma desorption mass spectra of NY.

Nystatin; positive ions, m/z 505~1,429, 2 nanoseconds/channel, 20,000 seconds.



Nystatin; negative ions, m/z 505~1,430, 2 nanoseconds/channel, 20,000 seconds.

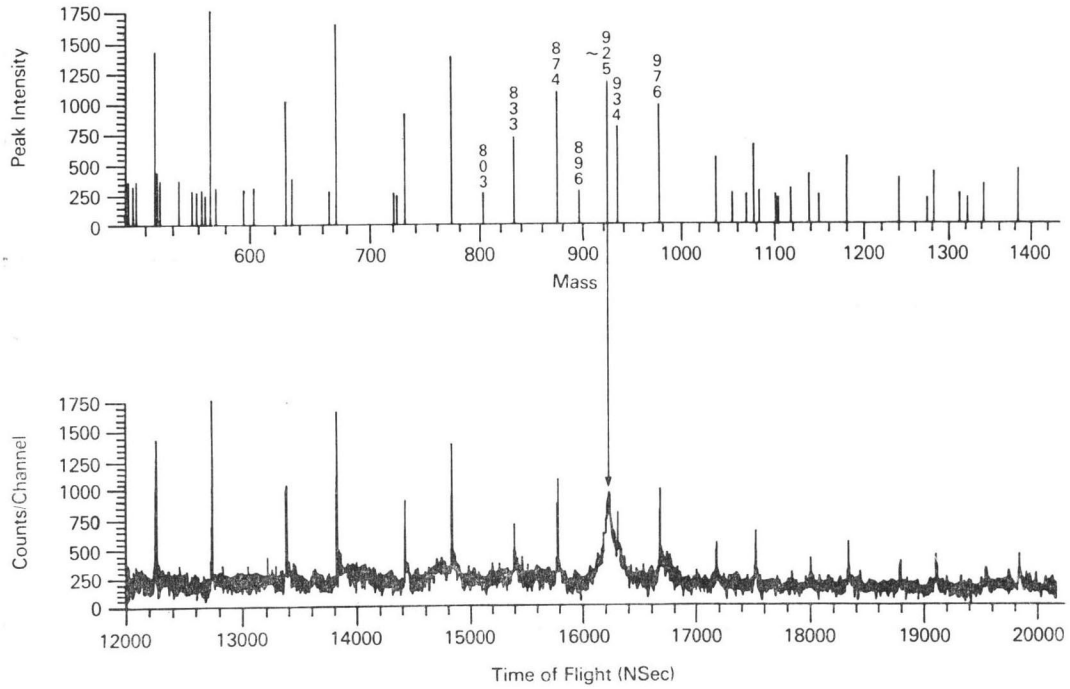
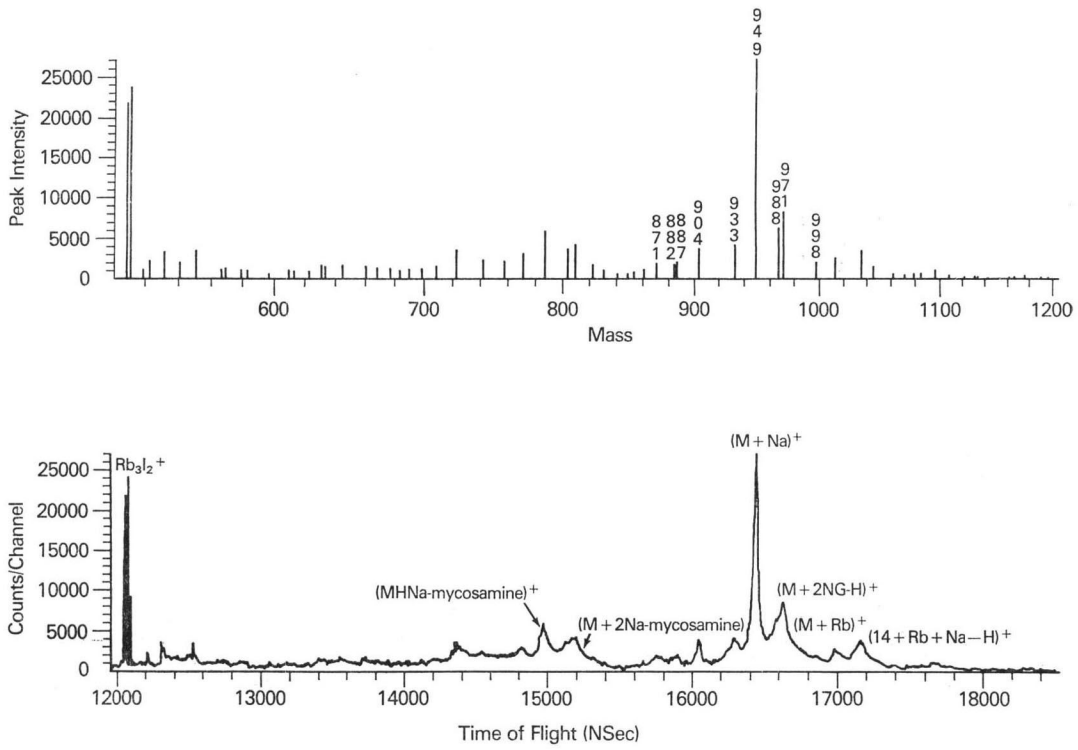


Fig. 3. Positive ion plasma desorption mass spectra of AA.

Amphotericin A on RbI; positive ions, m/z 501 ~ 1,205, 4 nanoseconds/channel, 36,000 seconds.



ring structure containing the ketal group. From the ions appearing below this m/z 348 can be assigned to ditrimethylsilyl-*N*-acetyl mycosamine and 332 to the same structure minus one oxygen. The fragmentation pattern appears to be somewhat different above m/z 404, but for both compounds the same molecular ion appears at m/z 1,759, corresponding to the structure of undecatrimethylsilyl-*N*-acetyl NY (hemiketal form).

Spectra obtained by PD-MS are in agreement with those of EI-MS. In the positive ion spectra of both NY (Fig. 2) and AA (Fig. 3), $(M+Na)^+$ ions appear at m/z 949 corresponding to a molecular ion of 926. Other ions which can be assigned are 787 and 809 in both spectra, which correspond to the aglycone portion of the molecules and their sodium adducts, respectively. The negative ion PD-MS of NY indicates M^- (Fig. 2) at m/z 926. AA did not give useful negative ion PD-MS. Spectra of the *N*-acetyl derivatives of these compounds obtained under the same conditions did not give any useful information.

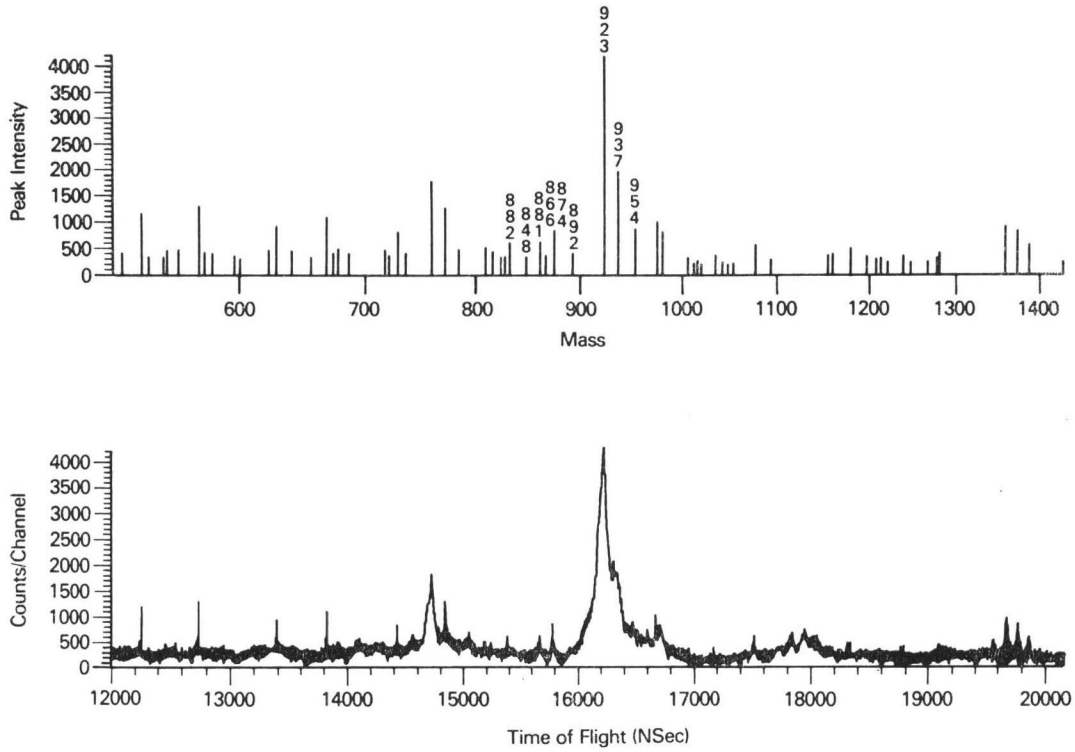
The positive ion PD-MS of AB (Fig. 4) gave $(M+Na)^+$ ion at m/z 947 and the negative ion PD-MS (Fig. 4) M^- at m/z 924, indicating one more unsaturation in the AB molecule compared to NY (and also to AA).

High Performance Liquid Chromatography

HPLC studies were performed as described in Materials and Methods, varying the acetonitrile concentration from 30 to 35% in 0.05 M phosphate buffer and the pH from 3.5 to 8.1. In each case, NY had a retention time 1 to 2 minutes longer than AA, except above pH 7.8, where AA had the

Fig. 4. Positive and negative ion plasma desorption mass spectra of AB.

Amphotericin B; negative ions, m/z 585~1,436, 2.80 nanoseconds/channel, 20,000 seconds.



Amphotericin B; positive ions, m/z 505~1,429, 2 nanoseconds/channel, 10,000 seconds.

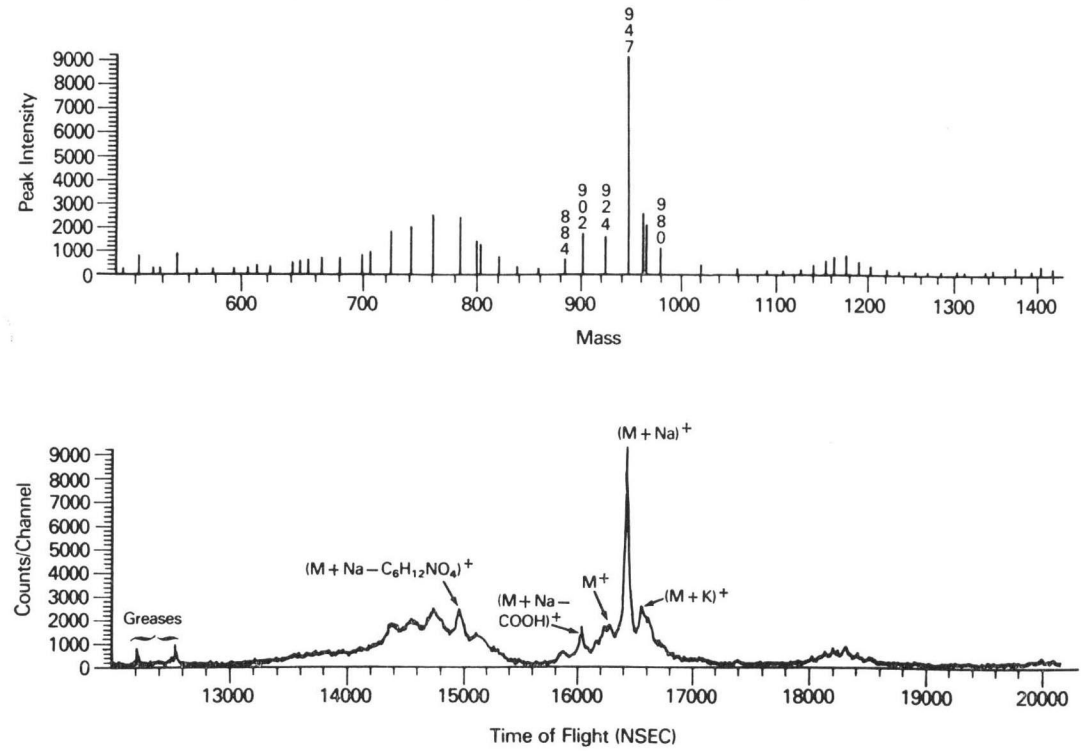


Table 2. Proton chemical shift assignments[a] in the 500 MHz NMR spectrum (DMSO-d_6, 60°C) of AB and AA.

Proton	Chemical shift (ppm)		Proton	Chemical shift (ppm)	
	AB	AA		AB	AA
2	2.18	2.32	29	—	2.10 m
3	4.07	4.36	30	—	5.52 dd
4	1.40	1.49	31	—	5.96
5	3.56	3.32	32	5.95, 6.50	5.96
6	1.36	1.48	33	5.46 d, d	5.52 dd
7	1.52	1.45	34	2.30 q, m	2.32 dd
8	3.13	3.28	35	3.20	3.13 d
9	3.48	3.55 m	36	1.75 d, d	1.83
10	1.34, 1.52	1.47, 1.54	37	5.21 d, d	5.08 m
11	4.27	4.24	38	1.14 d	1.12 d
12	1.57	1.62 d	39	0.92 d	0.87 d
13	—	—	40	1.05 d	0.97 d
14	1.15, 1.86	1.15, 1.89	1′	4.49	4.47 s
15	4.04	4.02 m	2′	3.65, 3.74	3.72 s
16	1.91	1.94	3′	2.75	2.68 d
17	4.19	4.02 m	4′	3.12	3.08 dd
18	1.55, 2.13	1.74, 1.80	5′	3.21 q, d	3.17 q
19	4.38	4.36 m	6′	1.18 d	1.16 d
28	—	2.14 m			

[a] When clear multiplets were observed they are indicated here. Two numbers at one carbon indicate that the two hydrogens on the same carbon are not equivalent.
d: Doublet, dd: doublet of a doublet, q: quartet, m: multiplet.

longer retention time (results not shown). In all systems, the two compounds had different retention times whether injected alone or co-injected.

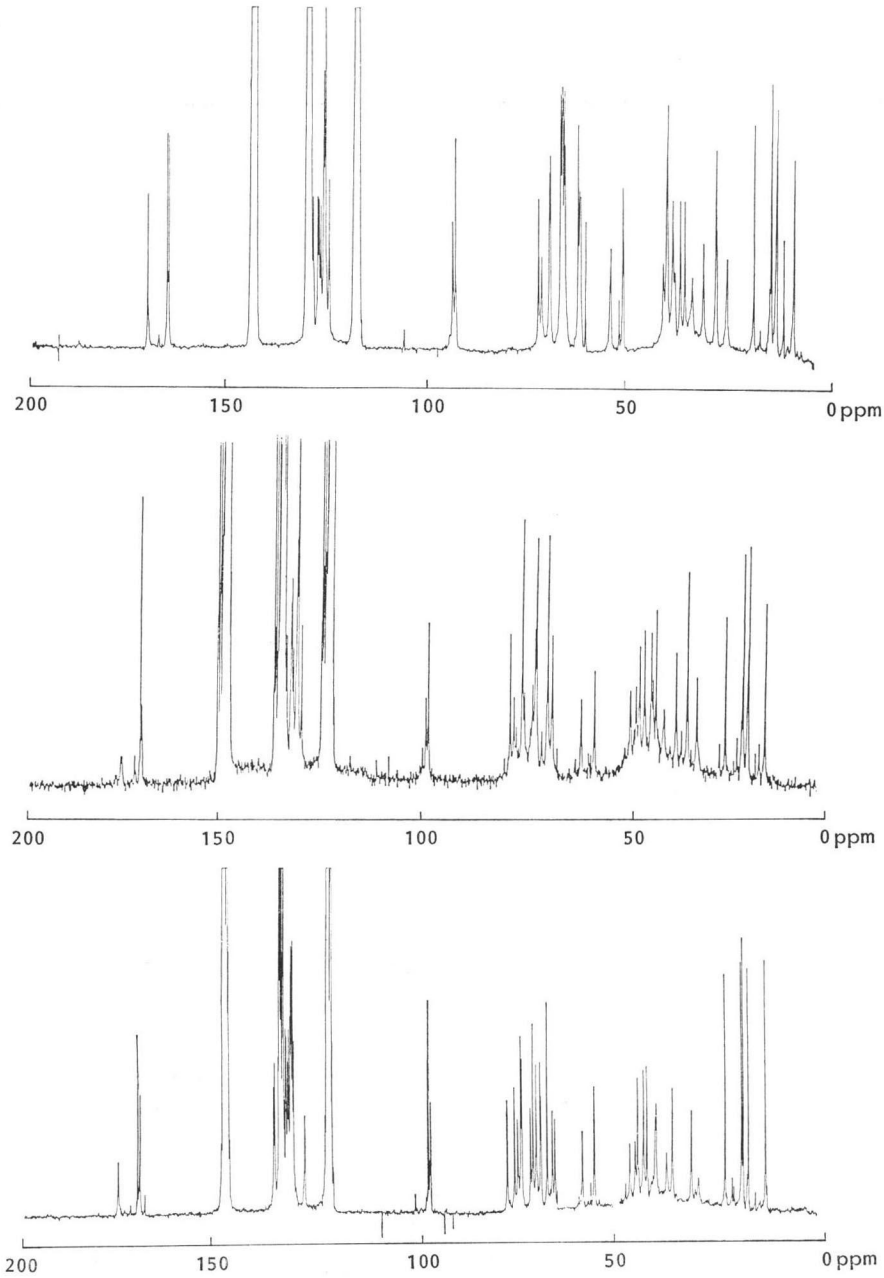
NMR Spectrometry

Proton shift assignments for the AB and AA molecules based on the 500 MHz 2D spectra are shown in Table 2. Proton shift assignments in the AB molecule had been made earlier with a 300 MHz instrument at 30°C, using NOE difference and spin decoupling difference techniques[23]. The assignments made based on that study and those reported here are in very good agreement. The slight differences may be due to the conformational difference of the AB molecule at two temperatures, 30 and 60°C. Some assignments that could not be made in the 300 MHz study, *i.e.*, H-6, H-26, H-28, H-31 and H-32, could be made in our study, mostly because of the 2D NMR technique and the 500 MHz field strength. Also, possible cross assignments were given for H-29 and H-30 in the 300 MHz study, while we could make these assignments unambiguously (see Table 2).

For illustrative purposes, the 200 MHz ^{13}C NMR spectra of *N*-acetyl NY, *N*-acetyl AA and *N*-acetyl AB are shown in Fig. 5. From the spectra, chemical shifts of 24 carbons could be assigned in the AA molecule (8, 9, 13, 16, 18, 20~27, 30~33, 34, 36, 39, 41, 1′, 3′ and 6′) and 26 in the NY molecule (those that are listed for AA and C-28 and C-29) (data not shown). These assignments match well with those obtained by the 500 MHz instrument and with literature values[24,25] and were confirmed by the INEPT technique (results not shown). The 200 MHz NMR spectrum of *N*-acetyl AB allows the assignment of 20 carbons as listed in Table 3.

The 2D NMR characteristics of AB were determined first because its structure is known[2]. Then

Fig. 5. 200 MHz ^{13}C NMR spectra of *N*-acetyl NY (upper), *N*-acetyl AA (middle) and *N*-acetyl AB (lower).



the same experimental conditions were used to obtain the 2D NMR spectral characteristics of AA. On the basis of the comparison of the 2D NMR characteristics of the two antibiotics, we propose the structure of AA as shown in Fig. 1.

The ^{13}C-^{1}H correlation maps of AB and AA are shown in Figs. 6 and 7, respectively. The ^{13}C chemical shift assignments are shown in Table 3.

The ^{1}H-^{1}H connectivity map for AB is shown in Fig. 8, marked with the connectivities for the

Table 3. ^{13}C Chemical shifts directly assignable in the AB and AA molecule by 500 MHz 2D NMR and in *N*-acetyl AB by 200 MHz NMR.

Carbon	*N*-Acetyl AB, 200 MHz (pyridine-d_5, 8°C)	AB, 500 MHz (DMSO-d_6, 60°C)	AA, 500 MHz (DMSO-d_6, 60°C)	Literature
1	171.8	—	—	173.2[24]
2	42.9	42.0	42.6	—
3	—	66.2	66.4	—
4	45.2	43.4	44.3	—
5	—	69.3	71.8	—
6	36.5	34.8	34.3	—
7	31.7	29.0	28.5	—
8	—	73.6	73.2	—
9	—	73.6	68.7	—
10	40.8	39.6	40.3	—
11	—	67.6	66.7	—
12	47.3	46.4	46.5	38.3~47.5[24]
13	98.3	—	97.0	98.4[24]
14	45.8	44.0	44.3	38.3~47.5[24]
15	—	65.4	66.1[a]	—
16	59.3	57.6	58.2	59.3[24]
17	—	65.2	65.7[a]	—
18	37.9	36.8	37.6	38.3~47.5[24]
19	—	74.6	75.5	—
20~27	—	—	—	—
28	—	—	31.8	—
29	—	—	31.7	—
30~33	—	—	—	—
34	41.7	42.0	40.8	—
35	—	77.0	76.2	—
36	40.5	39.7	40.2	—
37	—	69.0	70.4	70.1[24]
38	17.3	16.8	16.8	20.4[24]
39	12.7	11.8	12.2	11.0[25]
40	19.0	18.2	17.0	17.0[25]
41	176.6	—	—	—
1′	97.8	—	97.4	98.1[24]
2′	—	68.3	68.3	71.4~74.7[24]
3′	56.3	56.0	55.9	56.3[24]
4′	—	70.5	70.2	71.4~74.7[24]
5′	—	72.6	72.8	71.4~74.7[24]
6′	18.6	17.8	18.0	18.5[24]
Acetyl CH_3	23.1	—	—	23.1[24]
Acetyl C=O	171.3	—	—	171.4[24]

[a] Exchangeable assignments.

mycosamine region and for the region of C-33 to C-37. For comparative purposes, the ^1H-^1H correlation map of AA is shown in Fig. 9 marked with the connectivities for the same areas. The proton connectivities for AB and AA are similar, as can be seen in the ^1H-^1H correlation maps (Figs. 8 and 9). For the mycosamine moiety the two maps are identical. The C-19 to C-24 and C-26 to C-36 regions are also nearly identical. Some minor differences can be found because some cross peaks are very close together in the map of AA, while they are well separated in the map of AB. For example, cross peaks for H-21/22, H-22/23 and H-23/24 are very close together for AA (Fig. 9), but are separated for AB (Fig. 8). Major differences can be found between the correlation maps of AB and AA (Figs.

Fig. 6. ^{13}C-^{1}H correlation map of AB.

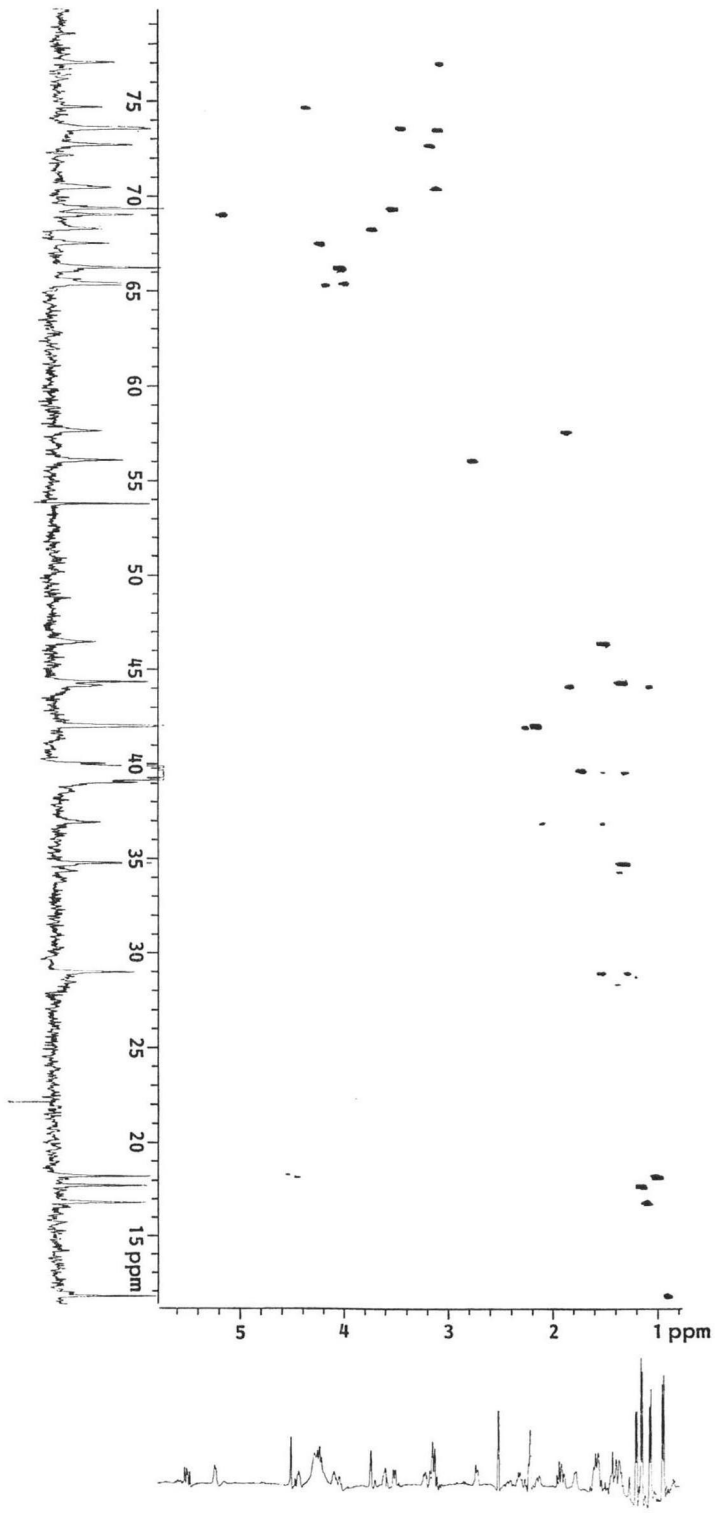


Fig. 7. ^{13}C-^{1}H correlation map of AA.

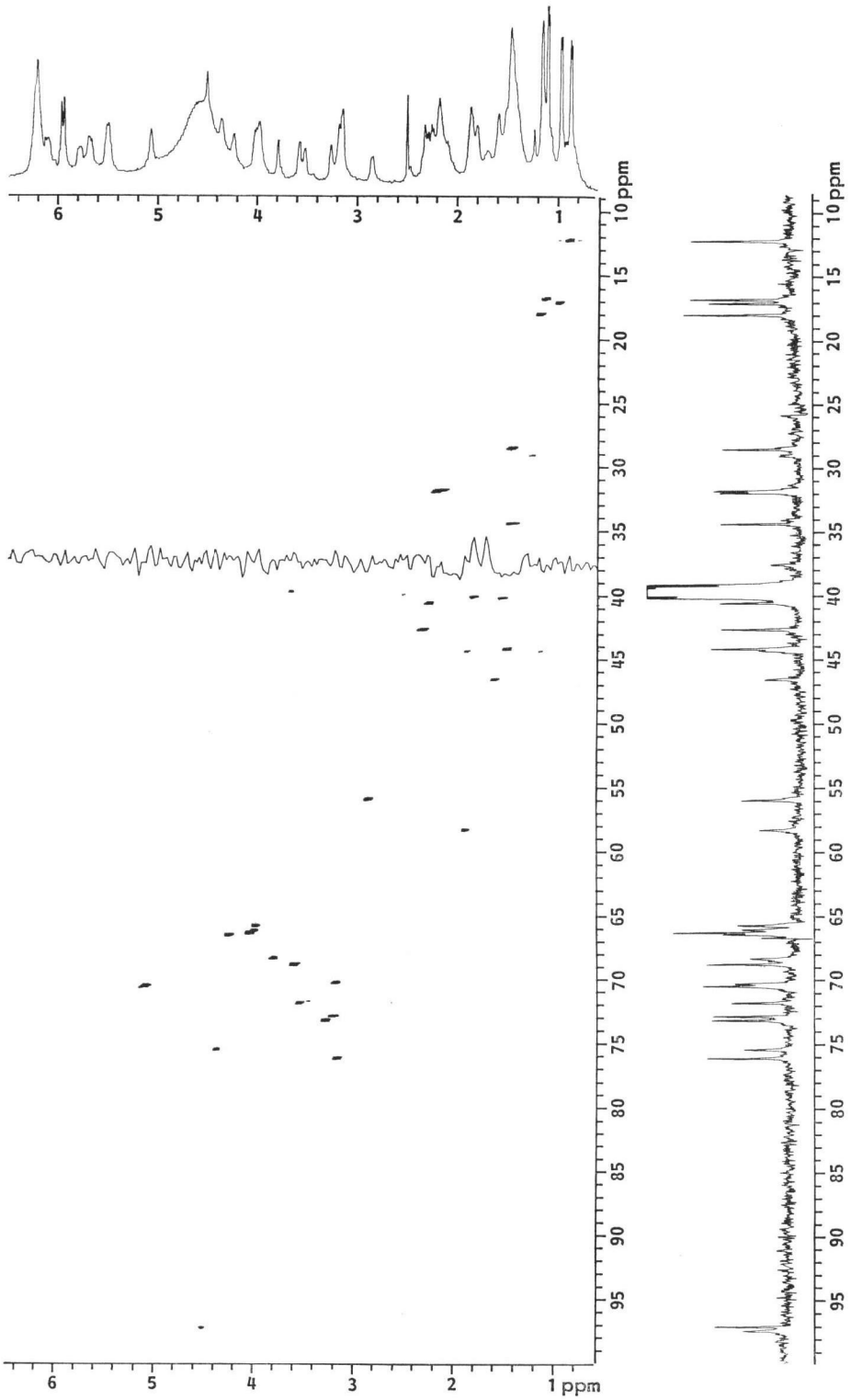
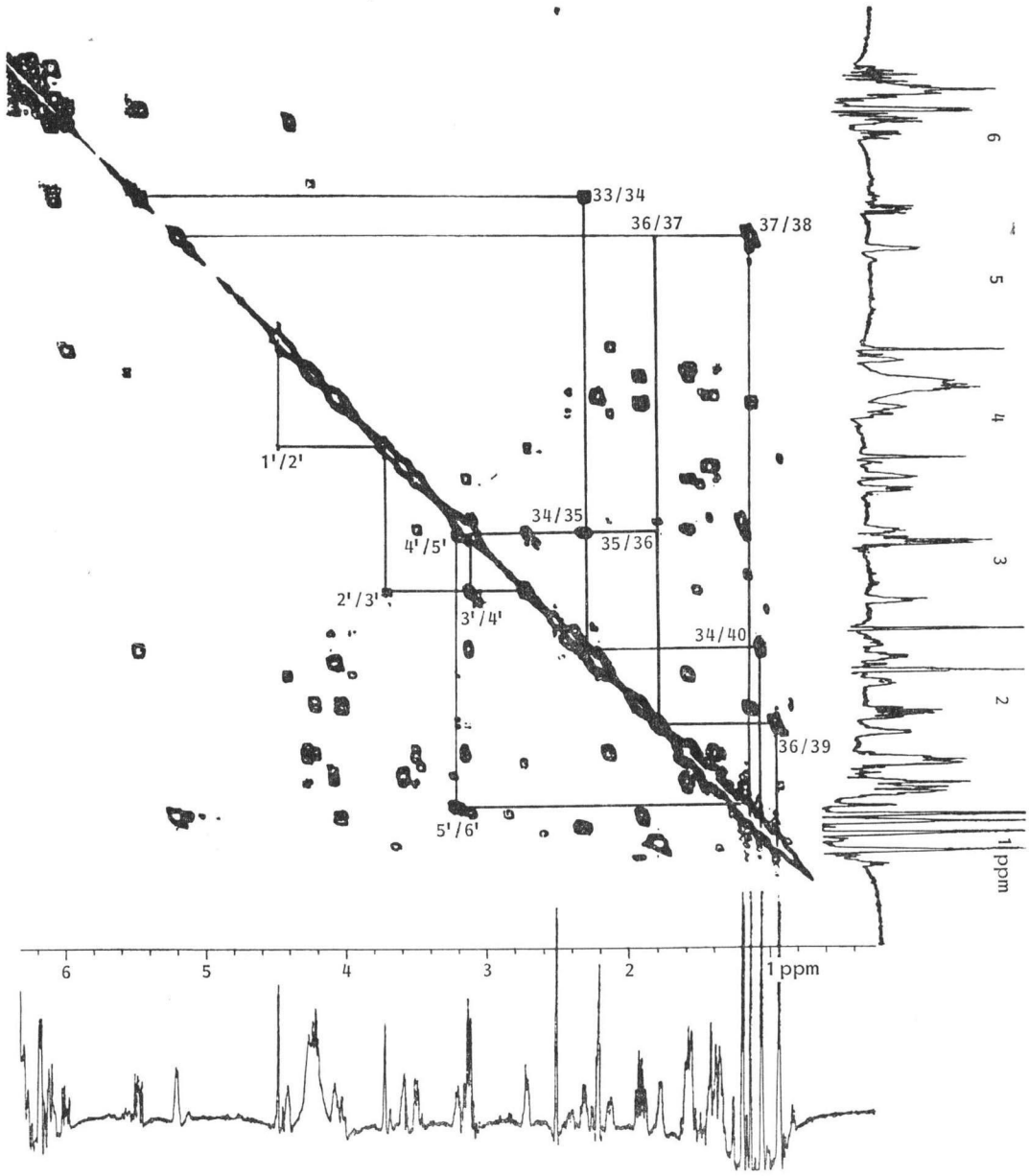
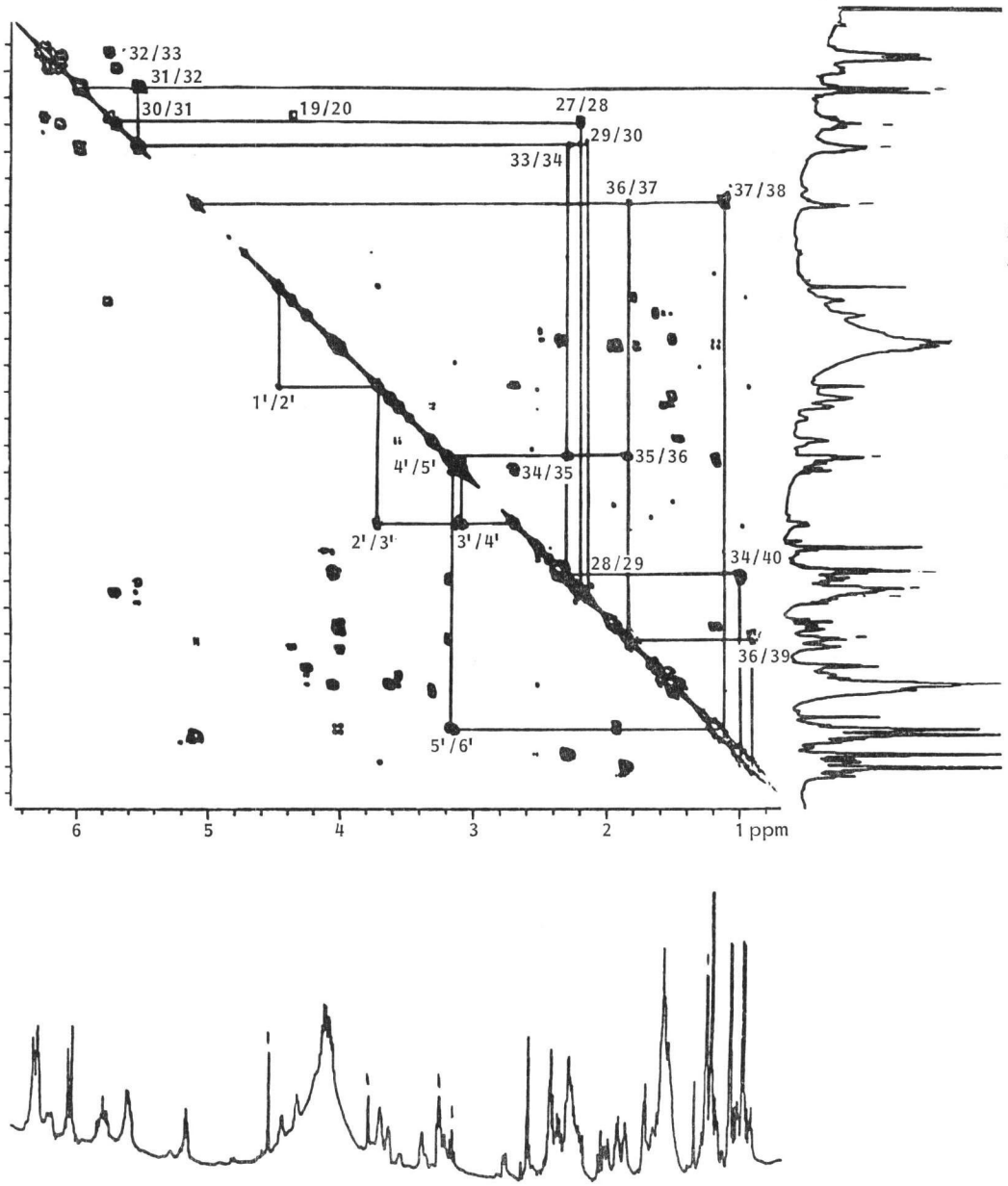


Fig. 8. ^{1}H-^{1}H correlation map of AB (C-33 to C-37 and mycosamine regions).



8 and 9, respectively), showing connectivities from H-19 to H-33. In the case of AB, the chemical shift of H-33 is at 5.46 ppm and the following connectivities all lie at this low field area, except that a cross peak can be found for H-19/20 (not marked in Fig. 8). Contrary to this, H-30 of AA (5.52 ppm) connects to high field methylene protons, H-29 and H-28 in the ^{1}H-^{1}H correlation map (Fig. 9). The chemical shift of H-29 is at 2.10 ppm and that of H-28 at 2.14 ppm. An interesting cross peak situation exists for the low field protons, *i.e.*, they overlap because some of these protons have the same chemical shift. For example, for AA the multiplets centered at 5.95 and 5.52 ppm each have an intensity corresponding to two protons (Fig. 9). In addition, the multiplet at 5.95 ppm has a structure

Fig. 9. ^{1}H-^{1}H correlation map of AA (C-34 to C-37 and mycosamine regions).



that is indicative of two coupled distinct hydrogens that have identical chemical shifts, *i.e.*, an AMM'X system[26]. The intense cross peak between these two multiplets confirms these assignments. Another difference between the ^{1}H-^{1}H correlation maps of AB and AA is that the cross peak H-36/37 can be seen clearly in the case of AA, while it is of very low intensity for AB[27].

Microbiological Assay

Results of the comparative microbiological assay of the two compounds are shown in Table 4. If both compounds are dissolved in DMSO initially, the activity against the three test organisms is

Table 4. MIC values (μg/ml) of NY and AA[a].

Antibiotic	Solvent	*C. albicans*	*C. tropicalis*	*S. cerevisiae*
NY	DMSO	0.45	0.32	0.35
	H_2O	0.39	0.21	0.23
AA	DMSO	0.80	0.40	0.35
	H_2O	10.1	5.21	3.5

[a] Test and strains of microorganisms are given in Materials and Methods.

about equal. However, aqueous suspensions of AA gave a higher MIC value than did NY. This latter result may be explained by the fact that different polymorphs dissolve at different rates in aqueous solvents.

Conclusions

We have concluded from the above study that NY and AA have the same molecular weight, 926; their microbiological characteristics are very similar. However, they can be separated by reversed phase HPLC. The ^{13}C 200 MHz spectra clearly indicate a difference between NY and AA, and allow the assignment of about half of the carbons in these molecules.

2D NMR studies have shown that the AA molecule is identical with that of AB except that there is a single bond between carbons 28 and 29. In an independent and simultaneous work, SOWINSKI *et al.*[28] recently reported similar results. However, in that work no ^{13}C-^{1}H correlation studies were done and no ^{13}C chemical shift assignments and no direct unambiguous ^{1}H assignments were made.

It is conceivable that the biosynthesis of AA partially follows that of NY, specifically in the region of the double bonds, and that of AB in other parts of the molecule.

References

1) VANDEPUTTE, J.; J. L. WACHTEL & E. T. STILLER: Amphotericins A and B, antifungal antibiotics produced by a streptomycete. II. The isolation and properties of the crystalline amphotericins. Antibiot. Annu. 1955/56: 587~591, 1956
2) MECHLINSKI, W.; C. P. SCHAFFNER, P. GAINS & G. AVITABILE: Structure and absolute configuration of the polyene macrolide antibiotic amphotericin B. Tetrahedron Lett. 12: 3873~3876, 1970
3) WALTERS, D. R.; J. D. DUTCHER & O. WINTERSTEINER: The structure of mycosamine. J. Am. Chem. Soc. 79: 5076~5077, 1957
4) CHONG, C. N. & R. W. RICKARDS: Macrolide antibiotic studies. XVI. The structure of nystatin. Tetrahedron Lett. 12: 5145~5148, 1970
5) SAFFERMAN, R. S.: Ph. D. Thesis, Rutgers Univ., 1959
6) SHENIN, Y. D.; T. V. KOTENKO & O. N. EKZEMPLYAROV: Composition of nystatin. Antibiotiki (Moscow) 13: 387~391, 1968
7) MARGOSIS, M. & A. ASZALOS: Quantitation of amphotericins by reverse-phase high-performance liquid chromatography. J. Pharm. Sci. 73: 835~838, 1985
8) MACFARLANE, R. D. & D. F. TORGERSON: Californium-252 plasma desorption mass spectroscopy. Science 191: 920~925, 1976
9) BURUM, D. P. & R. R. ERNST: Net polarization transfer *via* a *J*-ordered state for signal enhancement of low-sensitivity nuclei. J. Magn. Reson. 39: 163~168, 1980
10) AUE, W. P.; E. BARTHOLDI & R. R. ERNST: Two-dimensional spectroscopy. Application to nuclear magnetic resonance. J. Chem. Phys. 64: 2229~2246, 1976
11) BAX, A. & R. FREEMAN: Investigation of complex networks of spin-spin coupling by two-dimensional NMR. J. Magn. Reson. 44: 542~561, 1981
12) NAGAYAMA, K.; A. KUMAR, K. WÜTHRICH & R. R. ERNST: Experimental techniques of two-dimensional correlated spectroscopy. J. Magn. Reson. 40: 321~334, 1980
13) WIDER, G.; S. MACURA, A. KUMAR, R. R. ERNST & K. WÜTHRICH: Homonuclear two-dimensional ^{1}H NMR of proteins. Experimental procedures. J. Magn. Reson. 56: 207~234, 1984

14) BAX, A.: Two-Dimensional NMR in Liquids. Chapter 2, Reidel, Boston, 1982
15) DE MARCO, A. & K. WÜTHRICH: Digital filtering with a sinusoidal window function: an alternative technique for resolution enhancement in FT NMR. J. Magn. Reson. 24: 201~204, 1976
16) BAX, A. & S. K. SARKAR: Elimination of refocusing pulses in NMR experiments. J. Magn. Reson. 60: 170~176, 1984
17) MAUDSLEY, A. A.; L. MÜLLER & R. R. ERNST: Cross-correlation of spin-decoupled NMR spectra by heteronuclear two-dimensional spectroscopy. J. Magn. Reson. 28: 463~469, 1977
18) BODENHAUSEN, G. & R. FREEMAN: Correlation of proton and carbon-13 NMR spectra by heteronuclear two-dimensional spectroscopy. J. Magn. Reson. 28: 471~476, 1977
19) BAX, A. & G. A. MORRIS: An improved method for heteronuclear chemical shift correlation by two-dimensional NMR. J. Magn. Reson. 42: 501~505, 1981
20) BAX, A.: In Topics in Carbon-13 NMR Spectroscopy. Vol. 4, Chapter 8, John Wiley & Sons, New York, 1984
21) LINDON, J. C. & A. G. FERRIGE: Application of resolution enhancement to absolute value presentation of FT. Progr. Nucl. Magn. Reson. Spectrosc. 14: 27~44, 1980
22) Code of Federal Regulations, Title 21, 436.106, Apr. 1978
23) BROWN, J. M. & P. J. SIDEBOTTOM: The proton magnetic resonance spectrum of amphotericin B. Tetrahedron 37: 1421~1428, 1981
24) CEDER, O.; B. HANSSON & U. RAPP: Pimaricin-VIII. Structural and configurational studies by electron impact and field desorption mass spectrometry, ^{13}C (25.2 MHz) and ^{1}H (270 MHz)-NMR spectroscopy. Tetrahedron 33: 2703~2714, 1977
25) JOHNSON, L. F. & W. C. JANKOWSKI: Carbon 13 NMR Spectra. p. 483, John Wiley & Sons, New York, 1972
26) POPLE, J. A.; W. G. SCHNEIDER & H. J. BERNSTEIN: High-Resolution Nuclear Magnetic Resonance. Chapter 6, McGraw-Hill, New York, 1959
27) BAX, A.; R. A. BYRD & A. ASZALOS: Spin multiplet enhancement in two-dimensional correlated NMR spectroscopy. J. Am. Chem. Soc. 106: 7632~7633, 1984
28) SOWIŃSKI, P.; J. K. PAWLAK, E. BOROWSKI & T. IWASHITA: The structure of amphotericin A. II. The complete structure of the antibiotic. J. Antibiotics 38: 175~180, 1985