CHARACTERIZATION OF THE ANTIFUNGAL AND ANTIPROTOZOAL ANTIBIOTIC PARTRICIN AND STRUCTURAL STUDIES ON PARTRICINS A AND B

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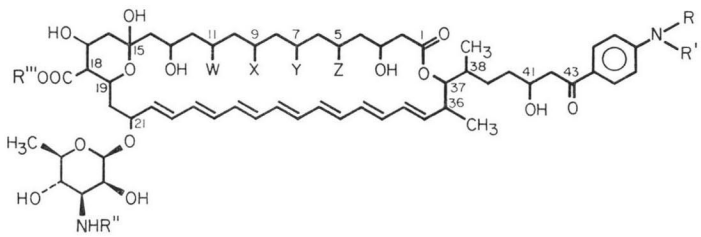
Partricin, a heptaene macrolide antibiotic, has been separated into three polyene components, partricins A, B and C, and one non-polyene component by countercurrent distribution. Treatment of partricin with base gave *p*-(methylamino)acetophenone and *p*-aminoacetophenone from partricins A and B, respectively, identifying both as members of the aromatic subgroup of the heptaene antibiotics. Both partricins A and B yield mycosamine on mild acid hydrolysis. NMR and mass spectral studies on products of ozonolysis or hydrogenolysis of acetyl derivatives provided evidence for the partial structures **1**~**9**.

Partricin, an antifungal and antiprotozoal antibiotic, is isolated from a strain of *Streptomyces aureofaciens* NRRL 3878[1]. Treatment of partricin with diazomethane gives the methyl ester[2], which retains good activity against *Candida albicans* and *Trichomonas vaginalis*, with considerably reduced mammalian toxicity compared to partricin. The methyl ester is marketed in several countries as Tricandil® for the treatment of vaginal infections. BRUZZESE and co-workers[1] identified partricin as a heptaene by the absorption peaks at 341, 359, 379 and 401 nm and found that it contains a carboxyl and two amino groups.



1: R=CH_3; R′=R″=R‴=H; W, X, Y, Z=$(OH)_3$, =O
2: R=R′=R″=R‴=H; W, X, Y, Z=$(OH)_3$, =O
3: R=CH_3; R′=R″=R‴=H; W, X, Y=$(OH)_3$, Z= =O
4: R=R′=R″=R‴=H; W, X, Y=$(OH)_3$, Z= =O
5: Same as **1** (=**3**) but R‴=CH_3
6: Same as **2** (=**4**) but R‴=CH_3
7: Same as **1** (=**3**) but R″=Ac
8: Same as **2** (=**4**) but R′=R″=Ac
9: Same as **1** (=**3**) but R′=R″=Ac

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We have separated partricin into its components by countercurrent distribution and in 1977 we described briefly evidence which assigned structures **1** and **2** to the major components, partricins A and B, respectively[3]. Later GOLIK *et al.* assigned the location of the keto group to C-5[4], thus completing the structural assignments for partricins A and B as **3** and **4**, respectively. More recently we reported the confirmation of molecular weights for partricins A and B by fast atom bombardment mass spectrometry[5]. We provide here details of the evidence which led us to the assignments of structures **1** and **2**.

Results

Partricin complex was separated (Fig. 1) by countercurrent distribution, using the system $CHCl_3$ - MeOH - borate buffer (pH 8.3, 0.05 M), 2: 2: 1[6], into three polyene components, partricins A, B and C (K_A=0.80, K_B=1.37, K_C=4.04) and one non-polyene (K=5.30). The properties of partricins A and B, the major components, and of C are found in Table 1. Partricin A, a greenish yellow powder, gave microanalyses agreeing with the formula $C_{59}H_{86}N_2O_{19}\cdot 4H_2O$ and partricin B, a brownish yellow powder, gave microanalyses agreeing with the formula $C_{58}H_{84}N_2O_{19}\cdot 2.5H_2O$. However, the molecular formulas assigned were placed on a firmer basis more recently by the fast atom bombardment spectra[7] of partricins A and B, which gave M+H ions at *m/z* 1,127 and 1,113, respectively, in the positive ion mode and

Table 1. Properties of partricins and their derivatives.

	Partricins			Acetyl derivatives	
	A	B	C	*N*-Acetylpartricin A	*N,N'*-Diacetyl-partricin B
Distribution coefficient (K)*	0.80	1.37	4.04		
Rf**	0.21,[a] 0.22,[b] 0.32,[c] 0.41,[d] 0.80,[e] 0.18,[f] 0.65[g]	0.19,[a] 0.17,[b] 0.27,[c] 0.39,[d] 0.78,[e] 0.16,[f] 0.65[g]		0.40,[a] 0.38,[b] 0.34,[c] 0.38,[d] 0.80,[e] 0.36,[f] 0.69[g]	0.35,[a] 0.33,[b] 0.28,[c] 0.36,[d] 0.76,[e] 0.33,[f] 0.69[g]
Color	Greenish yellow	Brownish yellow	Dark brown	Brownish yellow	Brownish yellow
Melting point	>300°C (dec)	>300°C (dec)		156~160°C (dec)	165~169°C (dec)
$[\alpha]_D^{26}$		+87.2°(*c* 0.06, DMF)			
UV λ_{max}^{MeOH} nm (ε)	400 (89,280) 378 (102,308) 358 (76,883) 342 (58,282) 288 (15,493) 247 (22,936) 240 (32,237) 232 (33,476)	400 (87,558) 378 (100,425) 358 (73,392) 342 (50,207) 288 (20,594) 247 (23,174) 240 (32,826) 232 (34,761)	400 378 358 340	400 (95,320) 378 (111,427) 358 (80,557) 340 (57,734) 287 (19,471) 246sh(25,509) 240 (34,912) 232 (35,577)	400 (97,175) 378 (110,881) 358 (78,493) 340 (47,338) 283 (34,887) 246sh(24,913) 240 (26,109) 232 (34,887)
Analysis (%)					
C	59.12, 58.78	59.85, 60.40		61.52	61.10
H	7.98, 7.51	7.89, 7.66		7.49	7.19
N	2.55, 2.36	2.57, 2.55		2.40	2.44
Elemental composition	$C_{59}H_{86}N_2O_{19}\cdot 4H_2O$	$C_{58}H_{84}N_2O_{19}\cdot 2.5H_2O$		$C_{61}H_{88}N_2O_{20}\cdot H_2O$	$C_{62}H_{88}N_2O_{21}\cdot H_2O$
Mol wt (FABMS)	1,127 (M+H),[†] 1,125 (M−H)[††]	1,113 (M+H),[†] 1,111 (M−H)[††]			
pK'a (in 70% DMF in H_2O)	6.07, 8.91	6.31, 8.95			6.90

* Solvent system: methanol - chloroform - borate buffer (pH 8.3, 0.05 M) (2: 2: 1),

** Solvent systems (*cf.* Experimental): [a]A, [b]B, [c]C, [d]D, [e]E, [f]F, [g]G.

[†] Positive ion mode. [††] Negative ion mode.

Fig. 1. Countercurrent distribution curve for partricin: MeOH - $CHCl_3$ - borate buffer (pH 8.3, 0.05 M), 2: 2: 1; n=600.

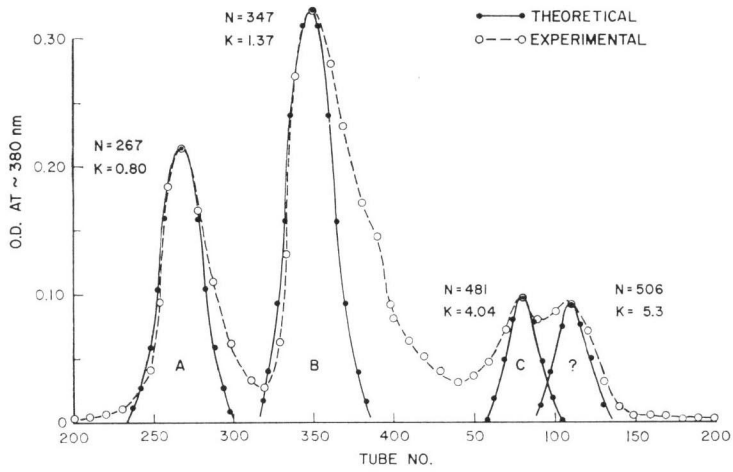


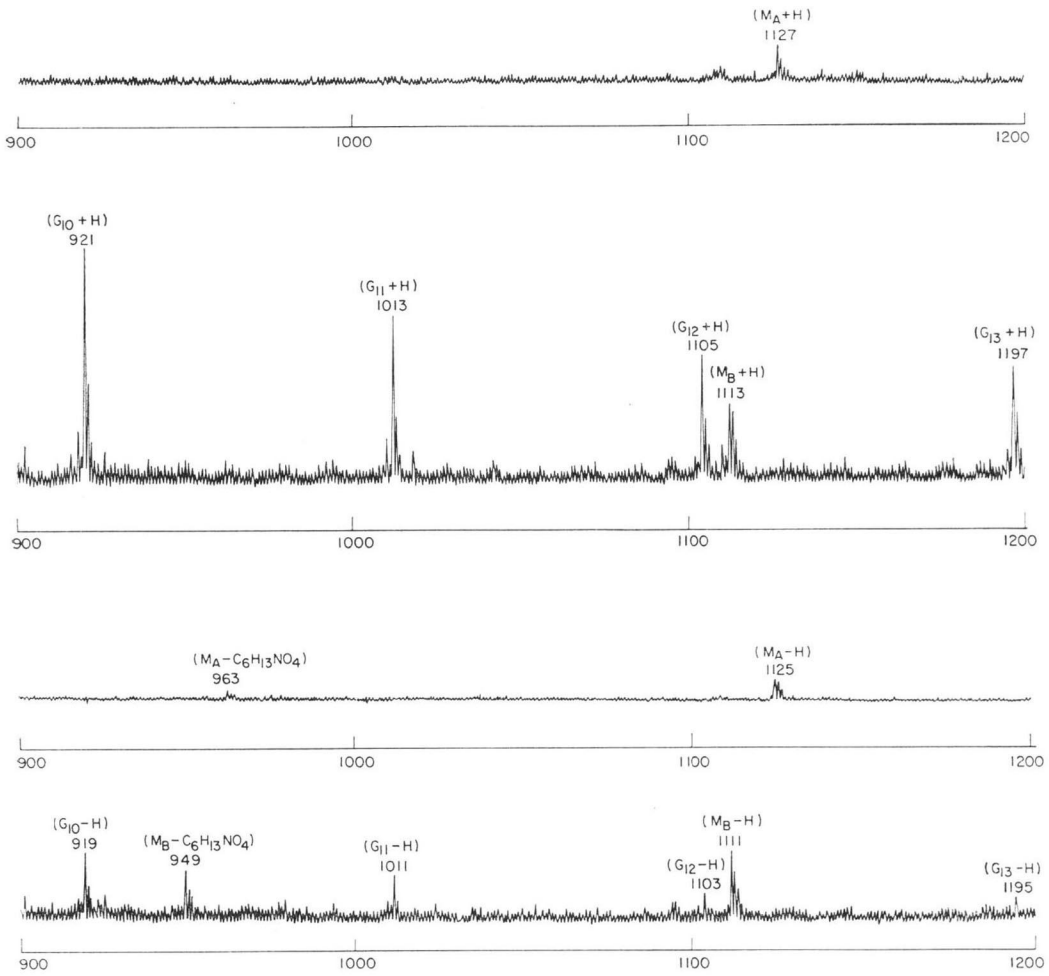
Table 2. Selected ^{13}C NMR signals in derivatives of partricins A and B and in model compounds.[a]

Assignments	δ[b,c]						
	N,N'-Diacetyl-partricin A (**9**)	*N*-Acetyl-partricin A (**7**)	*N,N'*-Diacetyl-tetradeca-hydro-partricin A (**14**)	*N,N'*-Diacetyl-partricin B (**8**)	Partricin methyl ester (**5, 6**)	*p*-Amino-aceto-phenone (**11**)[d]	*p*-(Methyl-amino)-aceto-phenone (**10**)[d]
>=O							
RCOR	208.1 s	208.1 s	208.3 s	208.5 s	208.2[d]		
ArCOR	198.5 s	196.4 s	198.5 s	198.2 s	196.6	196.5	196.4
COOH	174.1 s	174.2 s	174.4 s	174.6 s			
$COOCH_3$					173.1		
COOR (lactone)	170.1 s	170.1 s	170.7 s	170.3 s	170.2		
$ArNCOCH_3$	169.1 s		169.1 s	169.2 s			168.9
$NCOCH_3$ (mycosamine)	169.6 s	169.6 s	169.6 s	169.8 s			
Aromatic C's							
C-1 (attached to ketone)	135.4 s	125.0 s	135.4 s	132.1 s	130.5	127.6	131.5
ortho (to C-1)	129.3 d	130.4 d	129.3 d	129.6 d	125.1	130.8	129.4
meta (to C-1)	126.6 d	110.4 d	126.6 d	118.4 d	110.5	113.8	118.1
para (attached to nitrogen)	148.1 s	153.7 s	148.1 s	143.8 s	153.8	151.5	143.6
O- -C- O-							
Hemiketal	97.2 s	97.2 s	97.1 s	97.3 s	97.3		
Acetal	97.6 d	97.2 d	98.2 d		97.6		
$-CH_3$							
C–6′ (mycosamine)	18.0	18.0	18.1 q	18.1	17.9		
36–CH_3	16.4	16.4	15.8 q	16.4	16.4		
38–CH_3	12.9	12.8	13.3 q	12.9	12.8		
$ArNCH_3$	36.6	29.1	37.0 q				
$ArNCOCH_3$	22.4		22.4 q	24.1			24.1
$NHCOCH_3$ (mycosamine)	22.7	22.7	22.7 q	22.8			
$ArCOCH_3$						26.0	26.3
$COOCH_3$					51.4		

[a] For structures, see text. [b] ppm from TMS, DMSO-d_6 solutions. [c] Multiplicity in off-resonance spectra: s=singlet, d=doublet, q=quartet. [d] $CDCl_3$ solutions.

Fig. 2. Fast atom bombardment mass spectra: top and second, partricins A and B in positive ion mode; third and bottom, partricins A and B in negative ion mode.

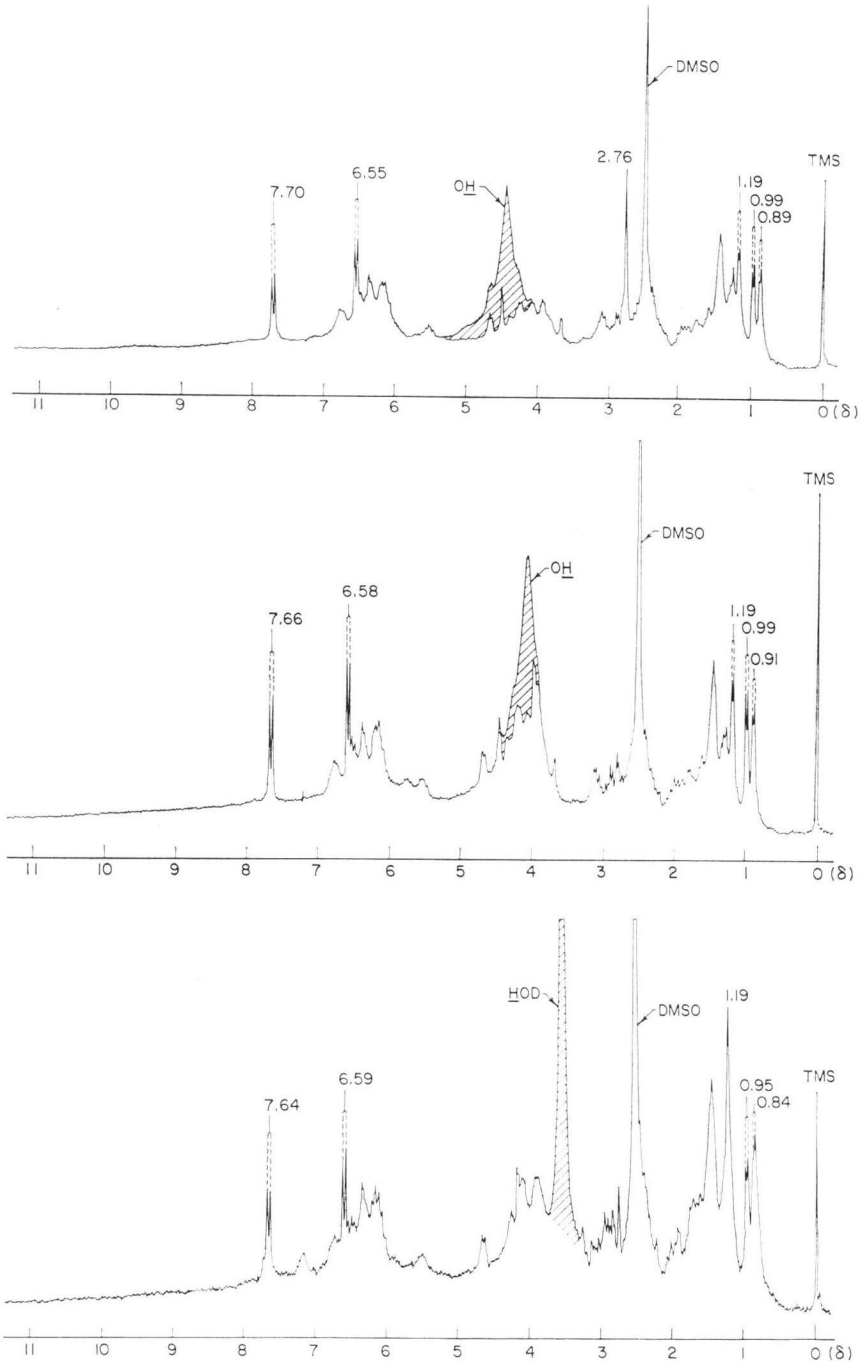
G_{10} refers to a cluster involving 10 moles of the glycerol matrix, *etc.* $C_6H_{13}NO_4$ is the molecular formula of mycosamine.



M − H ions at *m/z* 1,125 and 1,111 in the negative ion mode[5] (Fig. 2). Both the major components, partricins A and B, were converted to their methyl esters, **5** and **6**, respectively.

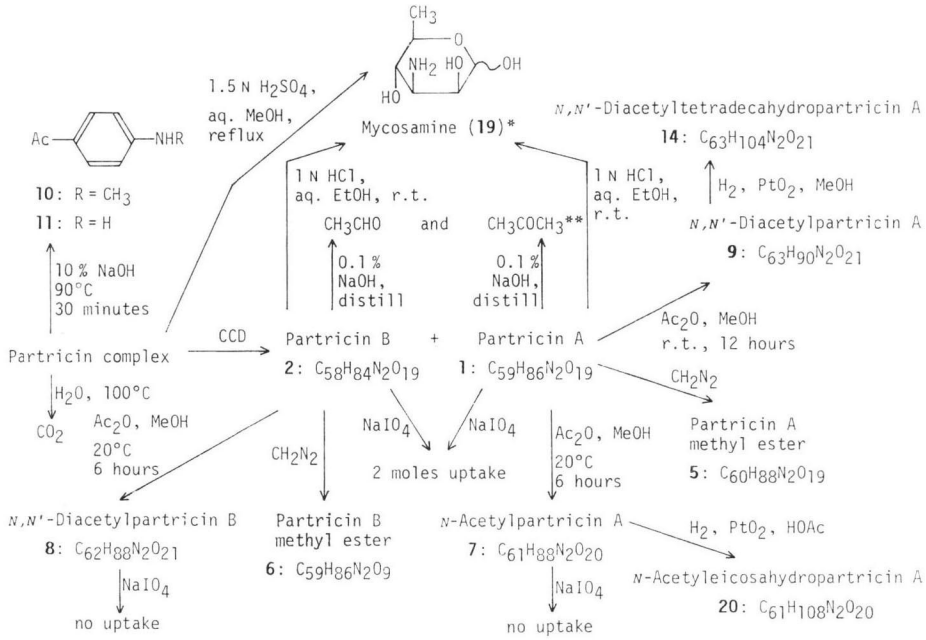
The molecular formulas suggested a difference of a methyl group and acetylation of partricin A in methanol - acetic anhydride gave an *N*-acetyl derivative (**7**), while B gave an *N,N'*-diacetyl compound (**8**). More vigorous acetylation conditions were required to give *N,N'*-diacetylpartricin A (**9**). This result argues for an *N*-methyl group in A lacking in B. In agreement with this hypothesis, the ^1H NMR spectrum of partricin A contained an *N*-methyl absorption at 2.76 ppm, lacking in the spectrum of partricin B (Fig. 3). Similarly, the ^{13}C NMR spectra (Table 2) of mono- and diacetylpartricins A (**7, 9**) contain absorptions at 29.1 and 36.6 ppm, respectively, lacking in the ^{13}C spectrum of diacetylpartricin B (**8**). This difference between partricins A and B was confirmed by basic hydrolysis of partricin complex with 10% sodium hydroxide at 90°C, which gave *p*-(methylamino)acetophenone (**10**) from partricin A and *p*-aminoacetophenone (**11**) from partricin B (Scheme 1) in a 1: 1 mixture.

Fig. 3. ^{1}H NMR spectra of partricins A (top), B and C (bottom) at 220 MHz.



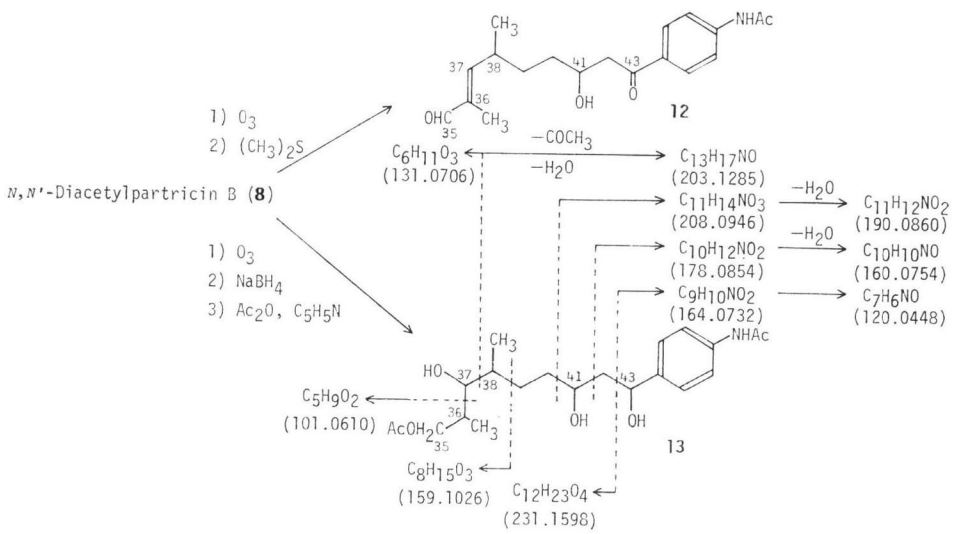
Isolation of **10** and **11** suggested on biosynthetic grounds that the sole difference between partricins A and B lay in the aromatic *N*-methyl group and this argument was supported by their acetyl derivatives' nearly identical ^{13}C NMR spectra (Table 2). Thus, structural studies on B could be used as evidence for

Scheme 1.



* Identified by TLC, GC and GC/EIMS as *N*-benzyloxycarbonyl and TMS derivatives.
** Identified as 2,4-dinitrophenylhydrazones.

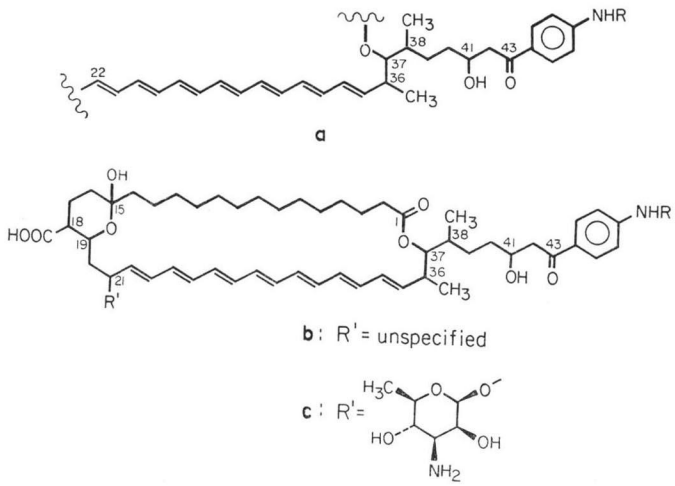
Scheme 2.



A and *vice versa*. The carbon skeleton of the partricins was extended by the results of ozonolysis, as shown next.

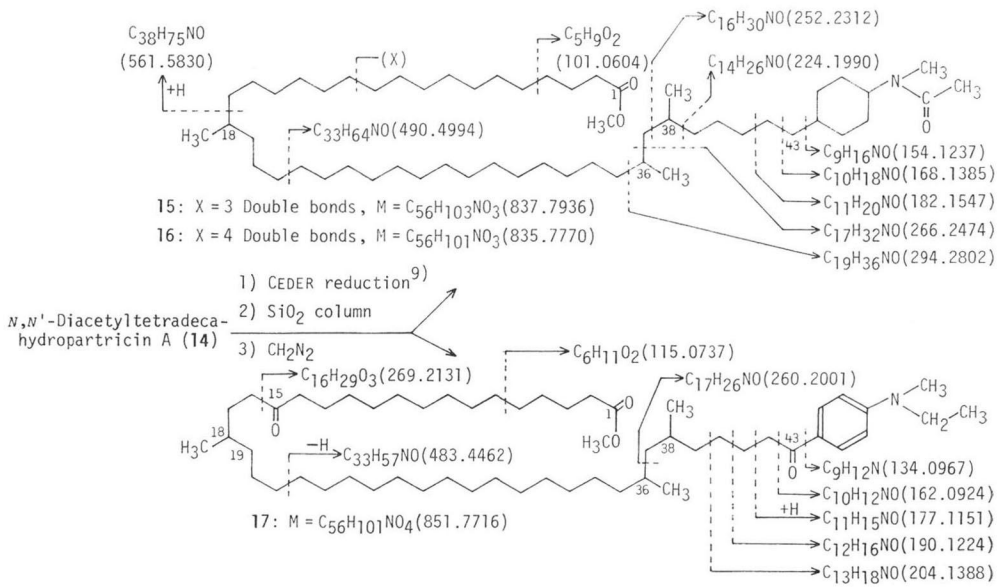
Ozonolysis of *N,N'*-diacetylpartricin B (**8**) at −75°C followed by dimethyl sulfide treatment and silica gel chromatography gave **12** (Scheme 2), whose structure was assigned during our earlier investigation

of hamycin[8], while ozonolysis, borohydride reduction and partial acetylation of **8** yielded **13**. The electron ionization (EI) mass spectrum of **13** had a molecular ion at *m/z* 395 as well as molecular ion-related peaks at *m/z* 377 (M − H_2O), 359 (M − $2H_2O$), 317.2034 ($C_{19}H_{27}NO_3$, M − H_2O − HOAc) and 299.1949 (M − $2H_2O$ − HOAc). Its fragmentation pattern, deduced from HRMS data (Scheme 2), and ^1H NMR spectrum are consistent with the assigned structure, based largely on the relationship of **13** to **12**. The ozonolysis products **12** and **13** establish the location of the heptaene unit and, in the absence of an olefinic methyl, establish the unit **a**.



The complete carbon skeleton of the partricins was then assigned by exhaustive reduction. CEDER reduction (Scheme 3)[9] of *N,N'*-diacetyltetradecahydropartricin A [**14**, obtained (Scheme 1) from *N,N'*-diacetylpartricin A (**9**) by hydrogenation over ADAMS platinum catalyst], followed by chromatography and methylation of the fractions with diazomethane, gave several fractions varying in polarity. One of

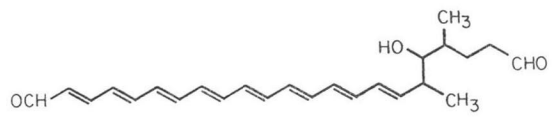
Scheme 3.



the fractions (Fraction 2 in Experimental) consisted of a pair of compounds, **15** and **16**, containing differing degrees of unsaturation, with molecular ions at *m/z* 837.7936 and 835.7770 ($C_{56}H_{103}NO_3$ and $C_{56}H_{101}NO_3$, respectively). These compounds thus contained the entire carbon skeleton of partricin A (C_{53}) plus the acetyl carbons (C_2) and a methyl ester (C_1). The mass spectrometric fragmentations (Scheme 3) clearly indicated a number of structural features directly related to the units already assigned [a reduced *p*-(*N*-methylacetamido)benzoyl group, methyl groups at C-38 and C-36], all located by the nitrogen marker (HRMS) as being near the *N*-terminus. The only other major nitrogen-containing fragment, $C_{38}H_{75}NO$, can be attributed to cleavage at a methyl, which must be on C-18. In intact partricin A, however, only three *C*-methyl groups are present [all doublets in the ^1H NMR spectrum (Fig. 3)], and they have already been located. Thus, the C-18 methyl must arise from reduction of the free carboxyl amino in **9**. On the other hand, the ion at *m/z* 101.0604 ($C_5H_8O_2$) lacks nitrogen and must arise from the C-terminal portion of the CEDER reduction product and this assigns a C-terminal carbomethoxy group with no unsaturation through C-4. Combining these fragments assigns structures **15** and **16** (Scheme 3).

The mass spectrum of another fraction (Fraction 1 in Experimental) gave a molecular ion with mass 851.7716, corresponding to the formula $C_{56}H_{101}NO_4$. Weaker molecular ions were found at *m/z* 849.7572 and 835.7770 ($C_{56}H_{99}NO_4$ and $C_{56}H_{101}NO_3$, respectively). In this case the aromatic ring is retained, as shown by the aromatic protons in the region 7~7.27 ppm of the ^1H NMR spectrum, and a carbomethoxyl group was observed at 3.65 ppm. Although the mass spectrum is complex, since it is that of a mixture, the ions indicated in Scheme 3 are in accord with the structural units shown, including keto functions at C-15 and C-43. Thus, structure **17** can be assigned.

The identification of hydrogenolysis products **15**, **16** and **17** assigns the complete carbon skeleton of partricin, confirms the methyl groups at C-36 and C-38, and locates the carboxyl at C-18 and a ketone at C-15. Since the ^{13}C NMR spectra of partricin derivatives (Table 2) indicate a ketal (or hemiketal) carbon, the ketone at C-15 is presumably in a hemiketal form, bonded to a hydroxyl at C-19, by analogy to the hemiketal of amphotericin B.[10] This placement would also correspond to that in hamycin A, which gave the octaenal **18** on treatment with base.[8] The unit **a** can now be extended to the partial structure **b**. Assignment of the lactone bond to C-37 is based on the isolation of **12** from elimination of the lactone during work-up of the ozonolysis.



18

The aminosugar mycosamine (**19**) was obtained by hydrolysis of partricins A and B with 1 N hydrochloric acid in 50% aqueous ethanol at 22°C for 2 hours and identified by TLC, GC and GC/EIMS. Its identity was further confirmed by its conversion to the *N*-benzyloxycarbonyl derivative, which was identical with an authentic sample obtained from nystatin.[11] The ease of hydrolysis of partricins A and B under mild conditions[12] indicates that the glycosidic bond is attached to a position allylic to the heptaene unit; thus, mycosamine is attached at C-21, as in unit **c**. The pyranose ring and β-glycosidic linkage of mycosamine are argued by the correspondence in ^{13}C NMR signals between the sugar methyl (C-6′) and acetal (C-1′) carbons of mycosamine in partricin (Table 2) and amphotericin B[10]. This extends partial formula **b** to **c**.

Both partricins A and B consumed 2 moles of sodium *meta*-periodate, the amount expected from cleavage of the mycosamine moiety alone, suggesting lack of periodate attack on the macrolide portion of the molecule (Scheme 1). This was confirmed when the *N*-acetylated derivatives (**7** and **8**) failed to react with periodate. Thus, there are no vicinal hydroxyl groups in the macrolide portion of partricins A and B. On the other hand, there must be an additional 6 hydroxyls in both partricins A and B, since partial formula **c** contains 12 oxygens, 7 less than the partricins, but only one additional element of unsaturation (the aliphatic keto group, *cf.* Table 2) is allowed, leaving no possibility of cyclic ethers.

The remaining 6 hydroxyls and the keto group cannot be at C-14 or C-16, and thus must be at C-3, C-5, C-7, C-9, C-11, C-13 and C-17, to avoid periodate cleavage (provided, of course, that there is no hydroxyl on C-20, which would be unlikely on biogenetic grounds as well as by analogy to hamycin and aureofungin B, which cleave to **18**).[8,13]

In keeping with the poly-β-hydroxy keto system, acetaldehyde and acetone were isolated as their 2,4-dinitrophenylhydrazones (in a ratio of 2~3 to 1) following steam distillation of a suspension of either partricin A or B in 0.1% aqueous sodium hydroxide (Scheme 1). Finally, the location of a hydroxyl (or ketone) on C-17 is in keeping with the observation that partricin lost carbon dioxide on heating in boiling water, arguing the presence of a δ-keto-β-hydroxy (or β-keto) acid.

Though a keto group at C-17 cannot be strictly excluded, it is highly unlikely, based on a) correspondence of the ^{13}C NMR hemiketal signal to that of amphotericin B (which also argues for a hydroxyl rather than a keto group at C-13) and b) the ratio of acetaldehyde to acetone from the base distillation. The argument based on the acetaldehyde - acetone ratio similarly discourages placing the keto group at C-3 or C-13 and the argument based on ^{13}C NMR signals assigns a hydroxyl to C-3 by comparison of the lactone absorptions of partricin derivatives with that of amphotericin B.[10] The nearly complete structures of partricins A and B are then assigned as **1** and **2**, respectively.

Relationship to Other Heptaene Antibiotics

Heptaene antibiotics are usually regarded as the most potent of the polyene antibiotics, providing the drug of choice (amphotericin B)[14] and others useful for the treatment of systemic fungal diseases as well as drugs of promise in the treatment of tumors[15] and viruses[16], benign prostatic hyperplasia[17] and in reduction of blood cholesterol levels[18]. Heptaenes can be divided into a non-aromatic group, which includes amphotericin B, and an aromatic group, which can be further subdivided according to the aminosugar constituent (mycosamine or perosamine)[19]. The present compounds, partricins A and B, belong to the mycosamine-containing aromatic heptaenes, a group which includes hamycins A and B[8], aureofungin A[13], vacidin A[20], gedamycin[21], ayfactin B[22], DJ400 B_1 and B_2[23,24], heptafungin A[25], X-63[26], trichomycin A[27], candicidin D (levorin A_2)[28], 67-121-A and C[29], and flavumycin A[30]. Of these, complete or partial structures have been assigned to hamycin A[8], aureofungin A[13], vacidin A[13], DJ400 B_1 and B_2[23,24], candicidin D[31], 67-121-A and C[29], and flavumycin A[30], and most of the structures are quite similar to that of hamycin A, the first structure to be assigned. In particular, they all share the same carbon skeleton and ring size, but differ in the number of keto, hydroxyl and *N*-methyl groups. MECHLINSKI and SCHAFFNER[32] found that they could separate most of the heptaenes by high pressure liquid chromatography (HPLC) on a Waters Associates μBondapak C_{18} column, employing a solvent system consisting of a mixture of acetonitrile - 0.05 M aqueous sodium citrate buffer (pH 5.3) in a ratio of 35: 65 or 32: 68, v/v. In their study they found that the chromatographic properties of partricin strongly suggested its identity with ayfactin. This relationship has been confirmed in the present study. Par-

tricin A migrates at the same rate in solvent B (Rf 0.22; *cf.* Experimental) as the faster moving component from ayfactin B (Bristol) and partricin B at the same rate as the slower moving component from ayfactin B. The FAB mass spectrum (positive ion) of one main ayfactin component contains an M+H ion at *m/z* 1,127 (like that of partricin A) and the FAB spectrum of the second main ayfactin component at *m/z* 1,111 (like that of partricin B).* Other possibly identical antibiotics are candicidin, levorin and heptamycin.[32]

Experimental

General Methods

Melting points were determined on a Kofler micro hot stage apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Beckman IR-12 spectrophotometer and electronic spectra were taken on a Beckman, Model CB, or Acta MVI recording spectrophotometer. Optical rotations were measured on a Zeiss polarimeter. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Varian HR-220 spectrometer equipped with a Nicolet TT220 Fourier transform accessory. Carbon magnetic resonance (^{13}C NMR) spectra were recorded either on a Varian XLFT-100 spectrometer with Digilab computer or on a JEOL, JNM-FX60 spectrometer. Proton and carbon chemical shifts (δ) are given as ppm relative to tetramethylsilane [$(CH_3)_4Si$] as internal standard. Low resolution mass spectra were obtained with a Varian MAT mass spectrometer, Model CH-5DF, employing a molecular beam inlet and E4B ion source. High resolution mass spectrometric measurements were made on a Varian MAT 731 mass spectrometer. Microanalyses were determined by Mr. J. NEMETH and associates. Gas chromatography was carried out with a Varian gas chromatograph, Series 1700, using a 1829×2 mm (i.d.) helical glass column packed with 3% OV-17 on Gas Chrom Q (100~120 mesh). Countercurrent distribution (CCD) was carried out on a 400-tube (10 ml/phase) automatic Craig instrument (H. O. Post Scientific Instrument Co., Inc., New York). Thin-layer chromatography (TLC) was carried out on Analtech precoated (250 μ) silica gel G TLC plates using the following solvent systems: A, 1-butanol - acetic acid - water (4: 1: 5, upper layer); B, chloroform - methanol - water - 30% ammonium hydroxide (64: 30: 4: 2); C, chloroform - methanol - borate buffer (pH 8.3, 0.05 M) (2: 2: 1, lower layer); D, 1-butanol - ethanol - water (4: 1: 5, upper layer); E, 1-butanol - ethanol - water (1: 1: 1); F, 1-butanol - acetic acid - water (7: 1: 1); G, 1-butanol - ethanol - acetone - 25% ammonium hydroxide (2: 5: 1: 3). The spots were visualized either by exposure to iodine vapor, UV light, a ninhydrin spray, sulfuric acid or a 20% aqueous solution of a 1: 1 mixture of ammonium sulfate and ammonium hydrogen sulfate.

Isolation of Partricins A, B and C by CCD

Partricin complex (batch #SN-644, 14 g) was dissolved in 400 ml of upper and 400 ml of lower phase of an equilibrated solvent system [methanol - chloroform - borate buffer (pH 8.3, 0.05 M), 2: 2: 1]. The insoluble material (~8 g) was filtered off and the filtrate was separated into lower and upper layers. Each layer was then loaded into tubes 0 to 39 (10 ml of each layer per tube) of the 400-tube CCD apparatus. The remaining tubes 40 to 400 were filled with 10 ml each of the lower layer and the central reservoir was filled with 4 liters of the upper layer. The connection between the reservoir and the first tube was adjusted so that during each transfer only 10 ml of the upper layer went into the first tube. The instrument was set for 20 shakes, 10 minutes settling time and 400 transfers, after which 10 μl samples were taken from every 10th tube and diluted to 5 ml with methanol. Electronic spectra were determined and the tubes showing no heptaene were emptied and refilled with 10 ml each of the fresh upper and lower layers of the same solvent system. Tube 0 was disconnected from the reservoir and connected to tube 400 and the instrument was set for another 200 transfers, thus making a total of 600 transfers. Electronic spectra were again checked for every 10th tube (Fig. 1). Theoretical distribution curves based on various distribution coefficients (K_A 0.80, K_B 1.37, K_C 4.04, K_D 5.30) are also shown in Fig. 1. Solutions containing partricins A, B and C were removed separately from the machine and concentrated

* Pyrolytic GC data (K. RORIG and R. TWEIT, unpublished) as well as antifungal and antitrichomonal data (Dr. ROBERT MUIR, Searle Research and Development, unpublished) also agree with these relationships.

at reduced pressure below 40°C to *ca.* 150 ml. Most of the partricin precipitated, then was centrifuged, washed with fresh deionized water (100 ml×6) to remove the buffer, and dried in a vacuum desiccator.

The combined fraction from tubes 230~310 yielded 814 mg (~6%) of pure partricin A: K_A 0.80; greenish yellow powder; mp >300°C (dec); TLC, single spot in solvent systems A~G (*cf.* Table 1); UV (75% MeOH in DMF) λ_{max} (ε) 400 (89,280), 378 (102,308), 358 (76,883), 342 (58,282), 288 (15,493), 247 (22,936), 240 (32,237), 232 nm (33,476); IR (KBr) 3430, 2945, 1720, 1640, 1600, 1578, 1542, 1490, 1400, 1350, 1300, 1182, 1140, 1110, 1075, 1045, 1005, 935, 850, 770 cm^{-1}; ^1H NMR (220 MHz, DMSO-d_6) 0.89, 0.99 and 1.19 (d's, J=7 Hz, >CHCH_3), 2.76 (s, >NCH_3), 6.55 and 7.70 ppm (d's, J=8 Hz, *p*-substituted Ar-*H*) (Fig. 3); pK_1 6.07, pK_2 8.91 (measured in ~70% DMF in H_2O); MS *m/z* 1,127 [M+H, fast atom bombardment mass spectrometry (FABMS), positive ion mode], 1,125 (M−H, FABMS, negative ion mode).

Anal. Calcd for $C_{59}H_{86}N_2O_{19}\cdot 4H_2O$: C 59.08, H 7.90, N 2.34.
Found: C 59.12, 58.78, H 7.98, 7.51, N 2.55, 2.36.

The combined fraction from tubes 320~400 yielded 980 mg (~7%) of pure partricin B: K_B 1.37; brownish yellow powder; mp >300°C (dec); $[\alpha]_D^{26}$ +87.2° (*c* 0.06, DMF); TLC, single spot in solvent systems A~G (*cf.* Table 1); UV (75% MeOH in DMF) λ_{max} (ε) 400 (87,558), 378 (100,425), 358 (73,392), 342 (50,207), 288 (20,594), 247 (23,174), 240 (32,826), 232 nm (34,761); IR (KBr) 3440, 2940, 1720, 1638, 1600, 1575, 1400, 1320, 1300, 1180, 1135, 1110, 1075, 1045, 1005, 885, 850, 770 cm^{-1}; ^1H NMR (220 MHz, DMSO-d_6) 0.91, 0.99 and 1.19 (d's, J=7 Hz, >CHCH_3), 6.58 and 7.66 ppm (d's, J=8 Hz, *p*-substituted Ar-*H*) (Fig. 3); pK_1 6.13, pK_2 8.95 (measured in ~70% DMF in H_2O); MS *m/z* 1,113 (M+H, FABMS, positive ion mode), 1,111 (M−H, FABMS, negative ion mode).

Anal. Calcd for $C_{58}H_{84}N_2O_{19}\cdot 2.5H_2O$: C 60.14, H 7.74, N 2.42.
Found: C 59.85, 60.40, H 7.89, 7.66, N 2.57, 2.55.

The combined fraction from tubes 71~110 yielded 447 mg (~3.2%) of partricin C: K_C 4.04; dark brown powder; UV (MeOH) λ_{max} 400, 378, 358, 340 nm; ^1H NMR (220 MHz, DMSO-d_6) 0.84 and 0.95 (d's, J=7 Hz, >CHCH_3), 6.59 and 7.64 ppm (d's, J=8 Hz, *p*-substituted Ar-*H*), probably with a third doublet buried in the peak at 1.19 (Fig. 3).

Partricin A Methyl Ester (**5**)

A solution of diazomethane in tetrahydrofuran was added to a magnetically stirred suspension of partricin A (250 mg) in methanol (25 ml) until all antibiotic was dissolved[33]. After 2 hours at room temperature excess diazomethane was decomposed with a few drops of glacial acetic acid and the dark yellow solution was filtered. The filtrate was concentrated and the residue was precipitated from ether, centrifuged, washed with fresh ether and dried under high vacuum to yield 180 mg (71%) of lemon yellow powder: mp 145~149°C (dec); UV (MeOH) λ_{max} (ε) 400 (79,326), 377 (92,454), 357 (68,094), 339 (51,685), 325 sh (36,603), 287 (14,199), 248 sh (16,092), 240 (24,612), 234 (26,505), 204 nm (16,092).

Partricin B Methyl Ester (**6**)

The procedure used above to prepare partricin A methyl ester was followed to yield, from 250 mg of partricin B, 195 mg (77%) of lemon yellow powder: mp 154~158°C (dec); UV (MeOH) λ_{max} (ε) 402 (81,101), 379 (94,729), 359 (64,171), 340 (41,558), 325 sh (32,330), 285 (16,196), 248 sh (12,529), 240 sh (20,474), 233 (23,835), 204 nm (21,696).

N-Acetylpartricin A (**7**)

Acetic anhydride (0.25 ml) in 5 ml of methanol was added to a stirred suspension of partricin A (500 mg) in 50 ml of methanol at 0°C. After 30 minutes at 0°C and 6 hours at 20°C, most of the material had dissolved. The mixture was filtered, then concentrated *in vacuo* at 20°C. The residue was precipitated by addition of ether (300 ml) and the mixture was centrifuged. The solid was washed with ether (100 ml×2) and dried under high vacuum (0.1 mm) to yield 457 mg (91%) of brownish yellow powder: mp 156~160°C (dec); TLC single spot in solvent systems A~G (*cf.* Table 1); UV (MeOH) λ_{max} (ε) 400 (95,320), 378 (111,427), 358 (80,557), 340 (57,734), 342 sh (41,616), 287 (19,471), 272 sh (15,441), 246 sh (25,509), 240 (34,912), 232 nm (35,577); IR (KBr) 3420, 2940, 1720, 1650, 1600, 1540, 1380, 1300, 1180, 1130, 1110, 1070, 1005, 940, 850, 765, 720 cm^{-1}; ^1H NMR (220 MHz, DMSO-d_6) 0.87, 0.97 and 1.17 (d's, J=7 Hz, CHCH_3), 1.88 (s, COCH_3), 2.75 (bs, NHCH_3), 7.72 and 6.55 ppm (d's, J=8 Hz,

p-substituted aromatic ring); ^{13}C NMR (25.2 MHz, DMSO-d_6), see Table 2; EIMS *m/z* 106.0657 (Δ0.0 mmu, C_7H_8N), 134.0606 (Δ 0.0 mmu, C_8H_8NO), 149.0841 (Δ 0.0 mmu, $C_9H_{11}NO$).

Anal. Calcd for $C_{61}H_{88}N_2O_{20}\cdot H_2O$: C 61.70, H 7.64, N 2.36.
Found: C 61.52, H 7.49, N 2.40.

N,N'-Diacetylpartricin A (**9**)

Acetic anhydride (5 ml) in 20 ml of absolute methanol was added to a stirred suspension of partricin A (4.0 g) in absolute methanol (380 ml) at room temperature. The reaction mixture was stirred for 12 hours, then filtered and the filtrate was concentrated *in vacuo* to 50 ml. The residue was precipitated by addition of ether, filtered, washed with fresh ether (~50 ml) and ether - pentane (1 : 1, 25 ml) and dried under high vacuum to yield 4.0 g (93%) of yellow powder: mp 155~160°C (dec); IR (KBr) 3430, 2945, 1725, 1715, 1660, 1655 sh, 1645 sh, 1635 sh, 1600, 1385, 1185, 1140, 1070, 1005, 850, 765 cm^{-1}; ^{1}H NMR (220 MHz, DMSO-d_6) 0.86, 0.98 and 1.17 (d's, J=7 Hz, >CHCH_3), 1.84 and 1.86 (s's, acetyl CH_3), 3.18 (s, >NCH_3), olefinic protons between 5.0 and 7.0, 7.42 and 7.95 ppm (d's, J=8 Hz, *p*-substituted Ar-H); ^{13}C NMR (25.2 MHz, DMSO-d_6), see Table 2.

Anal. Calcd for $C_{63}H_{90}N_2O_{21}\cdot H_2O$: C 61.55, H 7.55, N 2.28.
Found: C 61.41, H 8.28, N 2.55.

N,N'-Diacetylpartricin B (**8**)

The procedure given above for the preparation of *N*-acetylpartricin A (**7**) yielded, from 500 mg of partricin B, 460 mg (91%) of a yellow powder: mp 165~169°C (dec); TLC, single spot in solvent systems A~G (Table 1); UV (MeOH) λ_{max} (ε) 400 (97,175), 378 (110,881), 358 (78,493), 340 (47,338), 342 (33,643), 283 (34,887), 272 (26,168), 246 sh (24,913), 240 (26,109), 232 nm (34,887); IR (KBr) 3440, 2942, 1720, 1650, 1600, 1540, 1410, 1380, 1320, 1270, 1220, 1180, 1070, 1005, 850, 770 cm^{-1}; ^{1}H NMR (220 MHz, DMSO-d_6) 0.88, 0.97 and 1.17 (d's, J=7 Hz, >CHCH_3), 1.87 (s, acetyl CH_3), 2.08 (s), 7.68 and 7.88 ppm (d's, J=8 Hz, *p*-substituted Ar-H), also see Fig. 3; ^{13}C NMR (25.2 MHz, DMSO-d_6), see Table 2; EIMS *m/z* 120.0449 (Δ 0.0 mmu, C_7H_6NO), 162.0556 (Δ 0.1 mmu, $C_9H_8NO_2$), 177.0790 (Δ 0.0 mmu, $C_{10}H_{11}NO_2$); *pKa* 6.90 (in ~67% DMF).

Anal. Calcd for $C_{62}H_{88}N_2O_{21}\cdot H_2O$: C 61.26, H 7.46, N 2.31.
Found: C 61.10, H 7.19, N 2.44.

N,N'-Diacetyltetradecahydropartricin A (**14**)

A solution of *N,N'*-diacetylpartricin A (**9**, 1.18 g) in methanol (50 ml) was added to a suspension of pre-reduced Adams platinum catalyst (500 mg) in methanol (200 ml). Hydrogenation was carried out at 24°C for *ca.* 12 hours, when hydrogen uptake ceased. The catalyst was filtered, the absence of any heptaenic chromophore was confirmed by the electronic spectrum, and solvent was removed. The residue was precipitated from ether, filtered, washed with fresh ether and ether - pentane (1 : 1) and dried under high vacuum to yield 621 mg of powdery material; mp 115~120°C (dec); UV (MeOH) λ_{max} 336 sh, 260, 204 nm; IR (KBr) 3450, 2940, 2865, 1725, 1655, 1645 sh, 1602, 1382, 1188, 1070 cm^{-1}; ^{1}H NMR (220 MHz, DMSO-d_6) 0.82 and 1.17 (d's, J=7 Hz, >CHCH_3), 1.24 [s, –(CH_2)$_x$–], 1.87 and 1.90 (s's, acetyl CH_3), 3.2 (s, >NCH_3), 7.41 and 7.97 (d's, J=8 Hz, *p*-substituted Ar-H) and no olefinic protons between 4.8~7.3 ppm; ^{13}C NMR (25.2 MHz, DMSO-d_6), see Table 2.

Anal. Calcd for $C_{63}H_{104}N_2O_{21}\cdot H_2O$: C 60.85, H 8.59, N 2.25.
Found: C 61.03, H 8.39, N 2.06.

N-Acetyleicosahydropartricin A (**20**)

N-Acetylpartricin A (**7**, 1.2 g) was hydrogenated over pre-reduced Adams platinum catalyst (500 mg) in 200 ml of glacial acetic acid at atmospheric pressure and 24°C for 42 hours. The catalyst was filtered and washed with fresh acetic acid and solvent was removed at reduced pressure. The residue was precipitated from ether, then filtered, washed with ether and dried, giving 0.870 g (71%) of *N*-acetyleicosahydropartricin A: mp 115~125°C (dec); TLC, single spot in solvent system A; UV (MeOH) λ_{max} 272, 332 sh nm; IR (KBr) 3440, 2940, 2860, 1730, 1720 sh, 1650, 1580, 1385, 1260, 1185, 1070 cm^{-1}; ^{1}H NMR (220 MHz, DMSO-d_6) 0.82 and 1.18 (d's, 6 H, J=7 Hz, >CHCH_3), 1.27 [s, –(CH_2)$_x$–], 1.84 (s, >NCH_3), 1.88 (s, acetyl CH_3), 7.40 (>NH), no olefinic or aromatic protons below 4.6 ppm.

Anal. Calcd for $C_{61}H_{108}N_2O_{20}$: C 61.59, H 9.15, N 2.35.
Found: C 61.60, H 9.38, N 2.40.

Acidic Hydrolysis of Partricin to Mycosamine

A. Partricin Complex: A mixture of 5.50 g of partricin complex, 35.0 ml of methanol, 46.5 ml of water and 3.5 ml of concentrated sulfuric acid was heated at reflux for 2 hours while 30 ml of methanol was distilled off and cooled overnight. The aqueous supernatant was decanted, neutralized with solid sodium carbonate, stirred while 1.0 ml of benzyloxycarbonyl chloride was added dropwise, then for 2 hours more. The solid was filtered, dried and triturated with methanol and the filtrate was heated to boiling and concentrated to give 304 mg (~21%) of *N*-carbobenzyloxymycosamine: mp 197~199°C, $[\alpha]_D$ −15.5° (MeOH). Similar treatment of nystatin gave authentic *N*-carbobenzyloxymycosamine, mp 197.0~200.5°C, $[\alpha]_D$ −15.5° (MeOH) [Ref.[11] mp 190~193°C, $[\alpha]_D^{23}$ −15.5° (*c* 3.0, MeOH)], NMR and IR spectra identical to the sample from partricin complex.

B. Partricin A: Partricin A (200 mg) was stirred with 1 N hydrochloric acid in 50% aqueous ethanol (50 ml) at room temperature for 2 hours. Solvent was removed at reduced pressure at ~30°C and the residue was triturated with distilled water (10 ml×4). The combined aqueous fraction was freeze-dried to yield a ninhydrin-positive foamy residue (47.8 mg). TLC showed a single ninhydrin-positive spot corresponding (by comparison to an authentic sample) to mycosamine (**19**) in solvent systems A (Rf 0.21), D (Rf 0.41), and F (Rf 0.25). The residue (2 mg) was converted to the TMS derivative and injected onto a GC column (8°C/minute programmed from 80°C to 300°C, helium flow rate 18 ml/minute). The retention time (10 minutes) was identical to that of an authentic mycosamine TMS derivative. The identity was confirmed further by GC/EIMS.

C. Partricin B: By the procedure given above partricin B (150 mg) yielded 31.2 mg of a foamy residue indistinguishable from mycosamine by TLC, GC and GC/EIMS.

Basic Hydrolysis of Partricin Complex to *p*-Aminoacetophenone and *p*-(Methylamino)acetophenone

Partricin complex (5 g) was heated in 100 ml of 10% sodium hydroxide for 30 minutes at 90°C. After it had cooled the mixture was diluted to 400 ml with water and extracted thrice with chloroform. The extracts were evaporated and the residue was triturated with ether to yield a solid material, mp 72~76°C, whose ^{1}H NMR spectrum (ratio of N–CH_3 to CO–CH_3 peaks) indicated it to be a 1:1 mixture of *p*-aminoacetophenone and *p*-(methylamino)acetophenone.

Retroaldol Reaction

A. Partricin A: A mixture of 0.5 g of partricin A in a solution of 0.5 g of sodium hydroxide in 0.5 liter of water was heated while 60 ml of water was distilled into a solution of 2,4-dinitrophenylhydrazine (0.05 g) in 10 ml of 2 N hydrochloric acid. The solid which formed (0.05 g) was separated by filtration; its ^{1}H NMR spectrum matched those of authentic 2,4-dinitrophenylhydrazones of acetone and acetaldehyde, while integration indicated a ratio of 2 to 3 moles of acetaldehyde to one of acetone. Based on 7 moles of volatile carbonyl compounds, this corresponds to a 12% yield; PANDEY and RINEHART[34] obtained 15% of 6 moles of acetaldehyde from chainin.

B. Partricin B: When treated in the same way, partricin B gave a mixture of 2,4-dinitrophenylhydrazones with an identical ^{1}H NMR spectrum.

Ozonolysis of *N,N'*-Diacetylpartricin B (**8**)

A. Dimethyl Sulfide Workup: Ozone (Welsbach ozonator, settings: pressure 5 lbs; flow 1; voltage 80) was passed for 12 minutes through a magnetically stirred suspension of *N,N'*-diacetylpartricin B (**8**, 1.0 g) in methanol (200 ml) which had been cooled to −75°C with ethanol - dry ice. The reaction mixture was purged with oxygen for 5 minutes and the system was flushed with nitrogen for 20 minutes. Dimethyl sulfide (6 ml) was added at the above temperature and the system was flushed again with nitrogen for 20 minutes. The solution was then stirred at −10°C for 2 hours, 0°C for 1 hour and at room temperature for 1 hour. Solvent was removed at reduced pressure and the residue was taken up in dry methanol (~10 ml). A saturated solution of anhydrous potassium carbonate in dry methanol (~80 ml) was then added (pH~11.0) and the reaction mixture was stirred for 2 hours at room temperature and neutralized with acetic acid. Solvent was removed at reduced pressure at ~30°C and the residue was triturated with chloroform (25 ml×3). The combined chloroform-soluble material was concentrated and chromatographed on a column of silica gel II, eluting with chloroform, chloroform - methanol (99:1), and chloroform - methanol (98:2). A TLC-pure compound was isolated from the chloroform -

methanol (98: 2) eluate and crystallized from chloroform - benzene to yield white crystals (**12**, 18 mg): mp 135~137°C; Rf 0.60 ($CHCl_3$ - MeOH, 98: 2); ^1H NMR (100 MHz, $CDCl_3$) 1.11 (3 H, d, J=7 Hz), 1.78 (3 H, d, J=1.5 Hz), 2.24 (3 H, s), 6.28 (1 H, m), 7.63 and 7.91 (2 H ea, d's, J=8 Hz), 9.40 ppm (1 H, s); EIMS m/z 331 (M^+), 207, 162, 125, 97 amu.

Anal. Calcd for $C_{19}H_{25}NO_4$: mol wt, 331.1783.
Found: mol wt, 331.1781 (HREIMS).

B. Sodium Borohydride Workup: Ozone was bubbled at 1.7 liters/minute for 90 minutes through a stirred solution of *N,N'*-diacetylpartricin B (**8**, 1.9 g) in dimethylformamide (25 ml) and methanol (275 ml). Excess ozone was flushed with nitrogen and an aqueous solution of sodium borohydride was added until no more gas evolved. Methanol was removed at reduced pressure, acetic acid was added and the remaining volatile material was removed at reduced pressure. The residue was dissolved in pyridine (20 ml), acetic anhydride (20 ml) was added, and after 1 hour the solution was poured onto ice and extracted with chloroform (7×). The combined chloroform extracts were evaporated and the residue (0.8 g) was chromatographed on a column of silica gel (Woelm, 80 g), which was washed with ethanol - benzene (5 then 10%). The product was eluted with 15% ethanol in benzene, solvent was removed, and the residue was triturated with chloroform and dried to give 70 mg of product (**13**): ^1H NMR (60 MHz, CD_3OD) 2.11 (CH_3CO), 7.32 and 7.56 ppm (2 H ea, d's, J=9 Hz, Ar-*H*); EIMS, HREIMS m/z 395 (M^+), 377 ($M-H_2O$), 359 ($M-2H_2O$), 317.2034 (Δ-4.3, $C_{19}H_{27}NO_3$, $M-H_2O-AcOH$), 299 ($M-2H_2O-AcOH$), 231.1598 (Δ 0.3, $C_{12}H_{23}O_4$), 208.0946 (Δ-2.6, $C_{11}H_{14}NO_3$), 207.0915 (Δ 2.0, $C_{11}H_{13}NO_3$), 206.0829 (Δ 1.3, $C_{11}H_{12}NO_3$), 203.1285 (Δ-2.4, $C_{13}H_{17}NO$), 188.0714 (Δ 0.3, $C_{11}H_{10}NO_2$), 186.0895 (Δ-2.3, $C_{12}H_{12}NO$), 178.0854 (Δ-1.3, $C_{10}H_{12}NO_2$), 162.0549 (Δ-0.5, $C_9H_8NO_2$), 161.0838 (Δ-0.2, $C_{10}H_{11}NO$), 160.0754 (Δ-0.8, $C_{10}H_{10}NO$), 159.1026 (Δ 0.5, $C_8H_{15}O_3$), 131.0706 (Δ-0.1, $C_6H_{11}O_3$), 120.0448 (Δ-0.1, C_7H_6NO), 101.0610 (Δ 0.8, $C_5H_9O_2$).

Periodate Oxidations

Reactions were carried out at 22°C in aluminum foil-wrapped flasks using standard solutions of sodium *meta*-periodate, sodium arsenite, iodine and starch prepared from reagent grade chemicals.

Sodium *meta*-periodate (0.01 M, 20 ml) and acetate buffer (pH 5.3, 1 ml) were added to exactly weighed samples (20~30 mg) and the volume was adjusted to 50 ml with *tert*-butyl alcohol. Periodate consumption was measured with 5-ml aliquots at various times from 15 minutes to 40 hours. Both partricins A and B consumed one mole of periodate immediately and a second mole slowly, whereas *N*-acetylpartricin A and *N,N'*-diacetylpartricin B were inert to periodate oxidation under these conditions.

Decarboxylation of Partricin Complex

Partricin complex (1 g) was stirred and heated at 100°C with 20 ml of water for 4 hours while nitrogen was bubbled through. The evolved gases were passed through a barium hydroxide solution, which was filtered after completion of the reaction to yield 0.1 g of barium carbonate (57%, based on 1 mole of carbon dioxide per mole of partricin).

CEDER Reduction of *N,N'*-Diacetylpartricin A

A mixture of 10.1 g of *N,N'*-diacetylpartricin A (**9**), 50 ml of dimethylformamide, and 100 ml of methanol was hydrogenated with 1 g of platinum oxide for 2 hours at 140 g/cm² and room temperature in a 500-ml Parr shaker. The catalyst was filtered off and the filtrate was concentrated under vacuum. The residue was triturated with water and vacuum dried to give 8.4 g of solid. A mixture of the solid, 100 ml of acetic acid and 1.0 g of platinum oxide was placed in the glass liner of a 1-liter rocking bomb. The bomb was heated at 300°C for 4 hours with rocking at 268 kg/cm². After cooling, the liner contents were filtered, the filtrate was concentrated, and residue was triturated with water and extracted with ether. The extracts were evaporated and the residue was dissolved in hexane, cooled, filtered, and evaporated again. The residue (0.9 g) was dissolved in 10% ethyl acetate - toluene and gradient eluted with ethyl acetate - toluene from a low pressure column containing 80 g of Woelm silica. Elution was followed by TLC and 2 fractions of principal interest were eluted, starting with 25% ethyl acetate in toluene and ending with 40%. After concentration the residues were treated with ethereal

diazomethane and analyzed by LRMS and HRMS.

Fraction 1: ^{1}H NMR (100 MHz, $CDCl_3$) 3.65 ($COOCH_3$), 7.0 to 7.27 ppm (Ar-*H*); HREIMS *m/z* 851.7716 (M, Δ−1.3, $C_{56}H_{101}NO_4$), 849.7572 (Δ−0.1, $C_{56}H_{99}NO_4$), 483.4462 (Δ 2.3, $C_{33}H_{57}NO$), 269.2131 (Δ 1.6, $C_{16}H_{29}O_3$), 260.2001 (Δ−1.2, $C_{17}H_{26}NO$), 204.1388 (Δ 0.1, $C_{13}H_{18}NO$), 190.1224 (Δ −0.7, $C_{12}H_{16}NO$), 177.1151 (Δ−0.1, $C_{11}H_{15}NO$), 162.0924 (Δ 0.6, $C_{10}H_{12}NO$), 134.0967 (Δ−0.2, $C_9H_{12}N$), 115.0737 (Δ−2.0, $C_6H_{11}O_2$).

Fraction 2: HREIMS *m/z* 837.7936 (Δ 0.2, $C_{56}H_{103}NO_3$), 835.7770 (Δ−1.5, $C_{56}H_{101}NO_3$), 561.5830 (Δ−1.8, $C_{38}H_{75}NO$), 490.4994 (Δ 0.8, $C_{33}H_{64}NO$), 294.2802 (Δ 0.6, $C_{19}H_{36}NO$), 266.2474 (Δ−1.0, $C_{17}H_{32}NO$), 252.2312 (Δ−1.5, $C_{16}H_{30}NO$), 224.1990 (Δ−2.3, $C_{14}H_{26}NO$), 182.1547 (Δ 0.3, $C_{11}H_{20}NO$), 168.1385 (Δ−0.3, $C_{10}H_{18}NO$), 101.0604 (Δ 0.2, $C_5H_9O_2$).

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References

1) BRUZZESE, T. & R. FERRARI: Partricin. U. S. Patent 3,773,925, Nov. 20, 1973
2) BRUZZESE, T.; I. BINDA, A. DI NARDO, G. GHIELMETTI & M. RIVA: Partricin methyl ester, a semisynthetic polyene antibiotic. Experientia 28: 1515~1516, 1972
3) TWEIT, R. C.; K. L. RINEHART, Jr. & R. C. PANDEY: The chemical characterization of the antifungal and antiprotozoal antibiotic, partricin. 17th Intersci. Conf. Antimicrob. Agents & Chemother., Abstracts No. 72, American Society for Microbiology, New York, NY, Oct. 12~14, 1977
4) GOLIK, J.; J. ZIELINSKI & E. BOROWSKI: The structure of mepartricin A and mepartricin B. J. Antibiotics 33: 904~907, 1980
5) RINEHART, Jr., K. L.; J. C. COOK, Jr., R. C. PANDEY, M. D. LEE, C. P. SCHAFFNER, M. BARBER, R. S. BORDOLI, R. D. SEDGWICK, A. N. TYLER & B. N. GREEN: Polyene antibiotics studied by fast atom bombardment mass spectrometry. 29th Ann. Conf. Mass Spectrom. & Allied Topics, Abstract WAMOA10, Minneapolis, MN, May 24~29, 1981
6) HATTORI, K.; H. NAKANO, M. SEKI & Y. HIRATA: Studies on trichomycin. IV. J. Antibiotics, Ser. A, 9: 176~181, 1956
7) BARBER, M.; R. S. BORDOLI, R. D. SEDGWICK & A. N. TYLER: Fast atom bombardment of solids (F.A.B.): a new ion source for mass spectrometry. J. Chem. Soc. Chem. Commun. 1981: 325~327, 1981
8) PANDEY, R. C. & K. L. RINEHART, Jr.: Structural studies on hamycin A, an antifungal heptaene antibiotic. 16th Intersci. Conf. Antimicrob. Agents & Chemother., Abstracts No. 41, American Society for Microbiology, Chicago, IL, Oct. 27~29, 1976
9) CEDER, O.; J. M. WAISVISZ, M. G. VAN DER HOEVEN & R. RYHAGE: Pimaricin. II. High pressure-high temperature hydrogenation studies. Acta Chem. Scand. 18: 83~97, 1964
10) PANDEY, R. C. & K. L. RINEHART, Jr.: Polyene antibiotics. VII. Carbon-13 nuclear magnetic resonance evidence for cyclic hemiketals in the polyene antibiotics amphotericin B, nystatin A_1, tetrin A, tetrin B, lucensomycin and pimaricin. J. Antibiotics 29: 1035~1042, 1976
11) DUTCHER, J. D.; D. R. WALTERS & O. WINTERSTEINER: Nystatin. III. Mycosamine: preparation and determination of structure. J. Org. Chem. 28: 995~999, 1963
12) PANDEY, R. C.; V. F. GERMAN, Y. NISHIKAWA & K. L. RINEHART, Jr.: Polyene antibiotics. II. The structure of tetrin A. J. Am. Chem. Soc. 93: 3738~3747, 1971
13) LEE, M. D. & K. L. RINEHART, Jr.: The structures of aureofungins A and B. 16th Intersci. Conf. Antimicrob. Agents Chemother., Abstracts, No. 40, American Society for Microbiology, Chicago, IL, Oct. 27~29, 1976
14) The choice of antimicrobial drugs. Med. Lett. 24: 21~28, 1982
15) BRAJTBURG, J.; G. MEDOFF & G. S. KOBAYASHI: Antitumor effects of polyene antibiotics. *In* Antitumor Compounds of Natural Origin: Chemistry and Biochemistry, Vol. II, A. ASZALOS, *Ed.*, pp. 129~143,

CRC Press, Inc., Boca Raton, Florida, 1981
16) BORDEN, E. C.; J. A. MCBAIN & P. H. LEONHARDT: Effects of amphotericin B and its methyl ester on the antiviral activity of polyinosinic: polycytidylic acid. Antimicrob. Agents Chemother. 16: 203~209, 1979
17) GORDON, H. W. & C. P. SCHAFFNER: The effect of polyene macrolides on the prostate gland and canine prostatic hyperplasia. Proc. Nat. Acad. Sci. U.S.A. 60: 1201~1208, 1968
18) SCHAFFNER, C. P. & H. W. GORDON: The hypocholesterolemic activity of orally administered polyene macrolides. Proc. Nat. Acad. Sci. U.S.A. 61: 36~41, 1968
19) HAMILTON-MILLER, J. M. T.: Chemistry and biology of the polyene macrolide antibiotics. Bacteriol. Rev. 37: 166~196, 1973
20) ZIMINSKI, T. & K. L. RINEHART, Jr.: Structural studies on vacidin A, an antifungal antibiotic produced by *Streptomyces aureofaciens*. 15th Intersci. Conf. Antimicrob. Agents Chemother., Abstracts No. 428, American Society for Microbiology, Washington, D. C., Sept. 24~26, 1975
21) ZIMINSKI, T.; J. ZIELINSKI, E. BOROWSKI & K. L. RINEHART, Jr.: Badania strukturalne antybiotyku aureofacyna. Proc. Jubiles Scient. Convention of Polish Chemical Society and Association of Chemical Industry Engineers and Technicians, Abstr. book Vol. 1, p. 83, Wroclaw, Poland, Sept. 12, 1979
22) KAPLAN, M. A.; B. HEINEMANN, I. MYDLINSKI, F. H. BUCKWALTER, J. LEIN & I. R. HOOPER: An antifungal substance (AYF) produced by a strain of *Streptomyces aureofaciens*. Antibiot. Chemother. 8: 491~495, 1958
23) BOHLMANN, F.; E. V. DEHMLOW, H.-J. NEUHAHN, R. BRANDT & B. REINICKE: Neue Heptaen-makrolide. 1. Charakterisierung und Abbau. Tetrahedron 26: 2191~2198, 1970
24) BOHLMANN, F.; E. V. DEHMLOW, H.-J. NEUHAHN, R. BRANDT & H. BETHKE: Neue Heptaen-makrolide. 2. Grundskelett, Stellung der funktionellen Gruppen und Struktur der Aglykone. Tetrahedron 26: 2199~2207, 1970
25) KALASZ, H.; V. SZELL, J. GYIMESI, K. MAGYAR, I. HORVATH & I. SZABO: Antibiotics produced by *Streptomyces*. IX. Heptafungin-A, a new heptaene macrolide antibiotic. Acta Microbiol. Acad. Sci. Hung. 19: 111~120, 1972
26) KANNAN, L. V.; A. W. KHAN, M. R. KUTTY & V. C. VORA: A new heptaene antibiotic (X-63) from a *Streptomyces* species. J. Antibiotics, Ser. A 20: 293~294, 1967
27) HATTORI, K.: Studies on trichomycin. VIII. Chemical structure of trichomycin. J. Antibiotics, Ser. B, 15: 39~43, 1962
28) BOSSHARDT, R. & H. BICKEL: Zur Kenntnis von Levorin A und Candicidin. Experientia 24: 422~424, 1968
29) WRIGHT, J. J.; D. GREEVES, A. K. MALLAMS & D. H. PICKER: Structure elucidation of heptaene macrolide antibiotics 67-121-A and 67-121-C. J. Chem. Soc. Chem. Commun. 1977: 710~712, 1977
30) SHENIN, YU. D. & L. F. KROUGLIKOVA: The structure of aglycon moiety of flavumycin A, aromatic heptaene antibiotic. Bioorg. Khim. 3: 259~267, 1977
31) ZIELINSKI, J.; H. BOROWY-BOROWSKI, J. GOLIK, J. GUMIENIAK, T. ZIMINSKI, P. KOLODZIEJCZYK, J. PAWLAK, E. BOROWSKI, YU. D. SHENIN & A. I. FILIPPOVA: The structure of levorin A_2 and candicidin D. Tetrahedron Lett. 1979: 1791~1794, 1979
32) MECHLINSKI, W. & C. P. SCHAFFNER: Characterization of aromatic heptaene macrolide antibiotics by high performance liquid chromatography. J. Antibiotics 33: 591~599, 1980
33) PANDEY, R. C. & K. L. RINEHART, Jr.: Polyene antibiotics. IX. An improved method for the preparation of methyl esters of polyene antibiotics. J. Antibiotics 30: 158~162, 1977
34) PANDEY, R. C.; N. NARASIMHACHARI, K. L. RINEHART, Jr. & D. S. MILLINGTON: Polyene antibiotics. IV. Structure of chainin. J. Am. Chem. Soc. 94: 4306~4310, 1972