THE STRUCTURE OF MEPARTRICIN A AND MEPARTRICIN B

Sir:

Mepartricin (syn. SPA-S-160)*, the methyl ester of the heptaene macrolide antibiotic partricin (syn. SPA-S-132), is known to exhibit antifungal and anti-protozoal activities[1]. Partricin itself was isolated from a strain of *Streptomyces aureofaciens* NRRL 3878 as an antibiotic complex which later on was separated into two major components, named partricins A and B by counter-current distribution[2] according to the method of BOSSHARDT and BICKEL[3]. RINEHART *et al.*[2] have established the partial structures of partricins A and B as shown in Fig. 1. In the proposed structures the position of the lactone bond between C_1 and C_{37} and of the hydroxyl group at C_3 had been only postulated by the comparison of the chemical shifts in the ^{13}C NMR with other polyene macrolides.

Mepartricin, also a complex mixture, has been separated into mepartricins A and B as the main components of the antibiotic complex. Both compounds have been isolated by HPLC in analytical and by LPLC in preparative scales**.

The data obtained in our laboratory enabled us to supplement the partial structures of partricins A and B and to postulate the complete structures of mepartricins A (**1**) and B (**2**). The structures of these antibiotics are shown in Fig. 2.

The molecular weights of the both antibiotics were determined by FDMS method as 1,140 for A and 1,126 for B. Prominent quasi-molecular $(M+Na)^+$ ions were created. These results were confirmed by FDMS data of NN'-diacetylmepartricins A and B which were obtained by treating mepartricins A and B with acetic anhydride in the presence of triethylamine in tetrahydrofurane - water (3: 1, v/v) solution. In this case $(M+Na)^+$ at *m/z* 1,247 and *m/z* 1,233 were observed for components A and B, respectively. In the spectra of all of these compounds ions at 14 a.m.u. higher than the above observed masses were also present. The relative intensities of these ions were less than 20%. This parallels the LPLC fractionation of mepartricins A and B which revealed the existence of byproducts in the amount of about 20%, probably created as the result of further interaction of diazomethane with the antibiotics.

Further structural information was obtained from the EI mass spectra of the permethoxyderivatives **3** and **4** shown in Fig. 3, which were formed from **1** and **2** respectively, by the following reaction sequence: hydrogenation in the presence of 10% palladium on barium sulphate, N-acetylation, reduction with lithium borohydride (for "H" version) or with lithium borodeuteride (for "D" version) and methylation with methyl iodide in the presence of sodium hydride in tetrahydrofurane. The main products were separated from

Fig. 1. The partial structure of partricin A and partricin B according to RINEHART *et al.*[2]

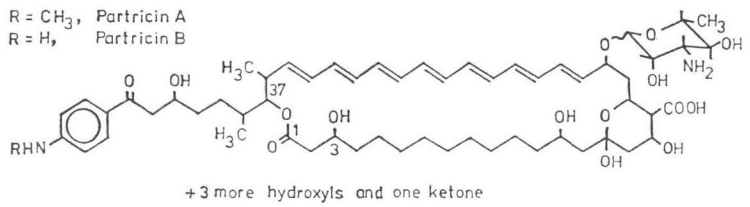
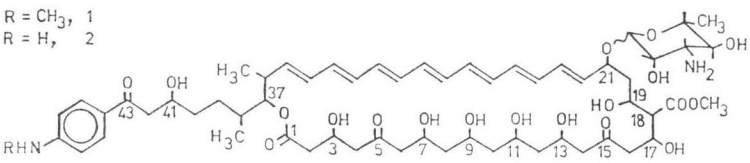


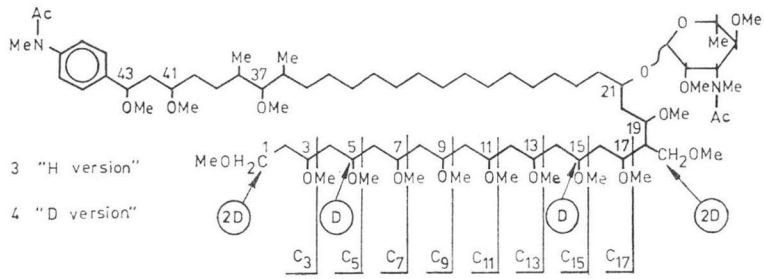
Fig. 2. The structure of mepartricin A (**1**) and mepartricin B (**2**).



* Mepartricin is marketed as "Tricandil" for the treatment of the vaginal infections by *Trichomonas vaginalis*.

** Separated mepartricins A and B by D. C. LIEBE from Searle Co. Laboratories, Chicago, Ill., have been used in our studies.

Fig. 3. The position of the oxygen functions in the carbon skeleton of mepartricin A and mepartricin B.



Assignment of ions	*m/z*	Relative intensity %	Elemental composition	*m/z*	Relative intensity %	Elemental composition
b	*101	21		101	22	
C_3	103.0737	17	$C_5H_{11}O_2$	105.0883	12	$C_5D_2H_9O_2$
C_5	129.0791	10	$C_7H_{13}O_2$	132.0969	11	$C_7D_3H_{10}O_2$
b	129.0918	29	$C_6H_4NO_2$	129.0912	43	$C_6H_4NO_2$
b	142	2		142	4	
C_7	155.1067	11	$C_9H_{15}O_2$	158.1275	8	$C_9D_3H_{12}O_2$
b	156	7		156	12	
a	176	27		176	47	
a	192	16		192	28	
b	201	28		201	43	
C_9	213.1515	2	$C_{12}H_{21}O_3$	216.1669	2	$C_{12}D_3H_{18}O_3$
b	230	100		230	100	
C_{11}	239.1648	3	$C_{14}H_{23}O_3$	242.1825	2	$C_{14}D_3H_{20}O_3$
b	246	4		246	10	
a	**254	1.9		254	24	
C_{13}	265.1825	3.4	$C_{16}H_{25}O_3$	268.1975	21	$C_{16}D_3H_{22}O_3$
C_{11}	271.1910	1.1	$C_{15}H_{27}O_4$	274.2087	8	$C_{15}D_3H_{24}O_4$
a	286	3.9		286	38	
C_{13}	397.2072	1.7	$C_{17}H_{29}O_4$	300.2247	13	$C_{17}D_3H_{26}O_4$
a	318	3.2		318	31	
C_{15}	323.2248	1.7	$C_{19}H_{31}O_4$	327.2471	7	$C_{17}D_4H_{27}O_4$
C_{17}	349.2386	1.6	$C_{21}H_{33}O_4$	353.2617	7	$C_{21}D_4H_{29}O_4$
C_{16}	355	0.7		359	2	
C_{17}	381.2655	1.5	$C_{22}H_{37}O_5$	385.2886	11	$C_{22}D_4H_{33}O_5$

Ions of relative intensities above * 6% and ** 1.2% have been tabularized.

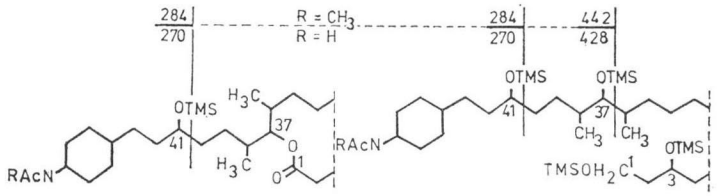
the reaction mixtures by chromatography on silica gel in a toluene - ethyl acetate - ethanol (10: 10: 1.8, v/v/v) solvent system.

The salient feature of the spectra of both compounds was the presence of molecular ions at *m/z* 1,458 or 1,464, respectively for "H" or "D" versions and the characteristic fragmentation ions, M−Me, M−(n×MeOH), M−(3-N-acetyl-N-methyl-2,4-O-dimethylmycosamine) and M−(3-N-acetyl-N-methyl-2,4-O-dimethylmycosamine) −n×MeOH. Diagnostic were also the prominent ions in the mass region below *m/z* 400 (Fig. 3). There were ions derived from fragmentation of the following parts of the molecule:

(1) 3-N-Acetyl-N-methyl-2,4-O-dimethylmycosamine at *m/z* 246, 230, 201, 156, 142, 129, 101 indicated in the spectra as "b"

(2) Carbon skeleton in the direction of the

Fig. 4. Determination of the position of the lactone bonds in mepartricin A and mepartricin B.



aromatic substituent at *m/z* 318, 286, 254, 192, 176 indicated as "a"

(3) Carbon skeleton at methoxyl functionalities from C_1 to C_{17} atoms

The latter fragmentation data enabled us to determine the position of remaining functional group in the molecule of both antibiotics.

Although the simple fragmentation ions were not observed (except *m/z* 103 for "H" version and *m/z* 105 for "D" version) they can be deduced from the ions arising after elimination of methanol molecules. HRMS measurements were made for the peaks of these series, and the results are shown on Fig. 3. Comparison of these data for both versions of polymethoxyols **3** and **4** analysed permitted the location of the hydroxyl groups in **1** and **2** at C_3, C_7, C_9, C_{11}, C_{13} and C_{17}. The positions and numbers of deuterium atoms in deutereo analogues indicated that the ketone functions were present at C_5 and C_{15}, while C_1 was the carboxyl carbon atom.

The position of the lactone bond was determined on the basis of HRMS measurements of the products which were formed from mepartricins A and B with the following reactions: Hydrogenation with 10% palladium oxide on barium sulphate until the aromatic ring absorption in the UV spectrum disappeared followed by N-acetylation with acetic anhydride in tetrahydrofurane - water (5: 1, v/v) solution. One part of the product was silylated with trimethylsilylimidazole, and another part was reduced with lithium borohydride and subsequently silylated with trimethylsilylimidazole. The products were analysed by HRMS, and the ions indicated in Fig. 4 were measured.

The ions at *m/z* 270.188 ($C_{14}H_{28}NO_2Si$) or *m/z* 284.2036 ($C_{15}H_{30}NO_2Si$) were present in the mass spectra of all compounds, but the ions at *m/z* 428.3016 ($C_{22}H_{46}NO_3Si_2$) or at *m/z* 442.3148 ($C_{23}H_{48}NO_3Si_2$) appeared only after reduction with lithium borohydride. This indicated that the hydroxyl groups at C_{37} of **1** and **2** interacted with the carboxyls at C_1 to form the lactone bonds.

The evidence presented above supplements the partial structure of partricins A and B proposed by RINEHART *et al*[2] and thus permits the postulation of the complete structure of these two antibiotics and their methyl esters. Both antibiotics have been reported by RINEHART *et al.* to form a secondary hemiketal structure involving C_{15} and C_{19}[2].

The structures of partricins A and B appeared to be identical with the constitution of main components of earlier isolated antibiotic aureofacin[4], namely gedamycin and vacidin A respectively[5].

Acknowledgements

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