POLYENE ANTIBIOTICS. V. CHARACTERIZATION OF COMPONENTS OF THE FILIPIN COMPLEX BY MASS SPECTROMETRY[1]

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A simple method has been developed for the characterization of neutral polyene antibiotics, involving conversion of the antibiotic to its polyacetate in acetic anhydride-pyridine. Mass spectra of these derivatives contain abundant molecular ions and the number of hydroxyl groups in the antibiotic can be inferred from the number of moles of acetic acid lost. Application of this method to filipins I, II, III and IV assigned them molecular formulas $C_{35}H_{58}O_9$, $C_{35}H_{58}O_{10}$, $C_{35}H_{58}O_{11}$, and $C_{35}H_{58}O_{11}$, respectively.

Recently, BERGY and EBLE[2] indicated that the pentaene antibiotic filipin described in the literature[3] is, in fact, a mixture which should be referred to as the filipin complex. Extensive work had already been carried out on the filipin complex on the assumption it was a single component[4,5,6] and a generally accepted structure (1) had been assigned.[7,8,9,10] BERGY and EBLE[2] were able to separate the filipin complex into filipins I, II, III, and IV, by column chromatography. All four components had the characteristic isolated pentaene chromophore. The last three were regarded as pure but filipin I was reported to be a complex.

Although properties of filipins I~IV were reported[2], including microanalyses, molecular formulas were not assigned, due to the difficulty in doing so without a limiting element such as nitrogen. For that matter, it has always been a problem to determine the exact molecular weights of polyene antibiotics, high molecular weight polyhydroxy compounds, and their formulas have undergone frequent revision for this reason. Recently, mass spectrometry has proved very useful in assigning molecular weights to antibiotics,[1,10,11,12] and the most common method now in use involves study of TMS derivatives[10,11]. In the present paper we wish to describe an alternative, very convenient, and versatile method for determining the molecular weights of polyene antibiotics by mass spectrometry, which we find often superior to trimethylsilylation since the derivatives themselves are stable to hydrolysis and give good molecular ions. This method has been successfully applied to other polyene antibiotics, and is reported here for tetrins I~IV.

We were limited especially by the small quantities of the individual components available, as little as 5 mg in some cases. Accordingly, the method involves treatment of *ca.* 5 mg of the antibiotic with acetic anhydride-pyridine to give a polyacetate. For most of the derivatives we have been able to observe the molecular ion at low

Table 1. Mass spectral peaks of filipin components

	Filipin [a]								
	I-H_{10}	II	II-H_{10}	III	III-H_{10}	IV	IV-H_{10}	F	F-H_{10}
P	926[b]	974[b]	984	1032[b]	1042	1032[b]	1042	1032	1042
c [c]		930	940	988		988		988	
P-HOAc	866	914	924	972	982	972	982	972	982
k-HOAc [d]	824		882	930	940	930	940	930	940
c-HOAc		870		928				928	
P-2HOAc	806	854	864	912	922	912	922	912	922
k-2HOAc	764	812	822	870	880	870	880	870	880
P-3HOAc	746	794	804	852	862	852	862	852	862
k-3HOAc	704	752	762	810	820	810	820	810	820
P-4HOAc	686	734	744	792	802	792	802	792	802
k-4HOAc	644		702	750	760	750		750	760
P-5HOAc	626	674	684	732	742	732	742	732	742
k-5HOAc	584		642		700	690	700	690	700
P-6HOAc	566	614	624	672	682	672	682	672	682
k-6HOAc	524		582		640	630	640	630	640
P-7HOAc	506	554	564	612	622	612	622	612	622
k-7HOAc					580		580		580
P-8HOAc				552	562		562	552	562
k-8HOAc					520				520
P-9HOAc				492	502			492	502

[a] I-IV=filipins I-IV; F=filipin complex; H_{10}=decahydro.
[b] Parent ion peak at 10.5~15 eV. [c] **c**=P-CO_2. [d] **k**=P-C_2H_2O.

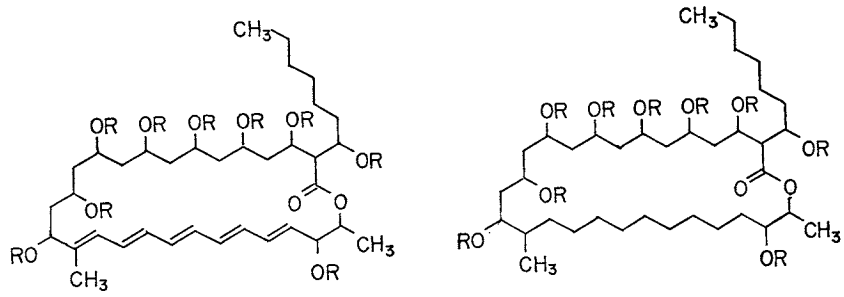
Table 2. Characteristics of filipin components

		Filipin			
		I	II	III	IV
Molecular formula [a)]		$C_{35}H_{58}O_9$	$C_{35}H_{58}O_{10}$	$C_{35}H_{58}O_{11}$	$C_{35}H_{58}O_{11}$
Hydroxyl groups [a)]		7	8	9	9
Double bonds [b)]		5	5	5	5
Calcd (found): [c)]	C	67.49 (66.97~67.24)	65.80 [f)] (63.69~63.93)	64.20 [f)] (61.01~62.14)	64.20 [f)] (61.82~61.94)
	H	9.39 (9.29~9.54)	9.15 (9.06~9.09)	8.93 (8.74~9.03)	8.93 (8.81~9.04)
	O	23.12 (23.52~23.85)	25.04 (25.71~25.82)	26.88 (27.40~27.50)	26.88 (27.66)
Rf [c,d)]		0.8	0.7	0.6	0.5
Partition chromatography [c,e)]		fastest	second	third	slowest

[a)] Based on mass spectra reported here.
[b)] Based on hydrogenation reported here.
[c)] Ref. 2.
[d)] Tlc, silica gel, CH_2Cl_2-MeOH (85 : 15).
[e)] Dicalite 436, DMF-H_2O-EtOAc-C_6H_{12} (10 : 1 : 25 : 50).
[f)] Carbon analyses (but not oxygen analyses) reported in Ref. 2 for filipins II, III and IV actually agree better with values calculated for hydrates. Calcd for $C_{35}H_{58}O_{10}\cdot H_2O$: C 64.00, H 9.21, O 26.80. Calcd for $C_{35}H_{58}O_{11}\cdot H_2O$: C 62.48, H 9.00, O 28.54.

ionizing voltage. Moreover, the number of hydroxyl groups in the original molecule can usually be determined by inspection of the fragmentation pattern, since each acetate unit is lost as acetic acid, giving a series of peaks from P-60 to P-60 n, where n is the number of hydroxyl groups. These characteristic peaks for the polyacetates of filipins II, III and IV are found in Table 1.

Fig. 1. Filipin (filipin III) and its derivatives.



1 : R = H
2 : R = $COCH_3$

3 : R = H
4 : R = $COCH_3$

To confirm the molecular weights and the number of hydroxyl groups, a 5-mg sample of each antibiotic was hydrogenated over platinum oxide in acetic acid and then the previous acetylation was repeated. Although, in general, a larger molecular ion peak was obtained for the non-hydrogenated polyacetates than the hydrogenated poylacetates, the mass spectra of the hydrogenated polyacetates showed molecular ions and indicated, in addition to the number of hydroxyl groups, the number of double bonds in the original molecule. Data for all the decahydro polyacetates of the filipin components (I, II, III and IV) have also been assembled in Table 1. Since the mass spectral properties of the filipin complex (Table 1) are close to those for filipin III and IV, these must be the major components of the complex, in agreement with the percentages reported: filipin I (4 %), II (25 %), III (53 %), IV (18 %)[2]. By the same token, since only one molecular weight was observed for filipin I, either a single component of that complex is predominant or the complex is composed of a mixture of isomers.

The molecular formula and number of hydroxyl groups assigned to filipin III agree with the structure assigned earlier,[7,8,9,10] to "filipin", the complex. The molecular formulas assigned the filipins indicate that filipin I has two hydroxyl groups fewer than filipin III and pilipin II has one hydroxyl fewer than III. Filipin IV is apparently isomeric with filipin III. These conclusions regarding structures of the filipin components agree well with the available data for the components (Table 2). Thus, the oxygen content of filipin III and IV is approximately equal and higher than that of II, which in turn is higher than I. Moreover, the additional hydroxyl groups in III and IV make those components slower moving than the others on both thin-layer and partition chromatographies.

Additional structural characterization of the filipin components will be described in a subsequent report. It is not yet clear why filipin II loses only seven moles of acetic acid in its mass spectrum, instead of the expected eight. Assignment of its complete structure, *i. e.* which hydroxyl of filipin III is missing, should clear up this point. One might predict that either the C-3 or the C-13 hydroxyl is lacking.

Experimental

Low resolution mass spectra were obtained by Mr. J. WRONA, on an Atlas CH4B mass spectrometer, employing a molecular beam inlet system.

Filipin Polyacetate. Filipins II, III, and IV (5-mg samples) were separately acetylated with excess acetic anhydride in pyridine for 12~18 hours at room temperature, then diluted with ice-water and extracted into chloroform. Work-up of the chloroform extracts gave the individual polyacetates of filipins II, III, and IV, whose mass spectral peaks are found in Table 1.

Decahydrofilipin Polyacetates. Filipins I, II, III, and IV (5-mg samples) were separately hydrogenated over platinum oxide in acetic acid at room temperature and atmospheric pressure. The mixtures were then filtered and evaporated and the residues were separately acetylated as above to give the individual decahydrofilipin polyacetates. Infrared spectra of the decahydrofilipin polyacetates differed very little, even in the fingerprint region. Mass spectral data are found in Table 1.

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Bibliography

1) PANDEY, R. C.; N. NARASIMHACHARI & K. L. RINEHART, Jr. : Polyene antibiotics. IV. The structure of chainin. Submitted.
2) BERGY, M. E. & T. E. EBLE : The filipin complex. Biochemistry 7 : 653~659, 1968
3) WHITFIELD, G. B.; T. D. BROCK, A. AMMANN, D. GOTTLIEB & H. E. CARTER : Filipin, an antifungal antibiotic. Isolation and properties. J. Amer. Chem. Soc. 77 : 4799~4801, 1955
4) SLONEKER, J. H. : The chemistry of filipin. Ph. D. Thesis, University of Illinois, Urbana, Ill., 1958
5) BERKOZ, B. & C. DJERASSI : Macrolide antibiotics. IX. Filipin. Proc. Chem. Soc. 1959 : 316~317, 1959
6) DJERASSI, C.; M. ISHIKAWA, H. BUDZIKIEWICZ, J. N. SHOOLERY & L. F. JOHNSON : The structure of the macrolide antibiotic filipin. Tetrahedron Lett. 1961(12) : 383~389, 1961
7) DHAR, M. L.; V. THALLER & M. C. WHITING : The structures of lagosin and filipin. Proc. Chem. Soc. 1960 : 310~311, 1960
8) DHAR, M. L.; V. THALLER & M. C. WHITING : Researches on polyenes. VIII. The structures of lagosin and filipin. J. Chem. Soc. 1964 : 842~861, 1964
9) CEDER, O. & R. RYHAGE : The structure of filipin. Acta Chem. Scand. 18 : 558~560, 1964
10) GOLDING, B. T.; R. W. RICKARDS & M. BARBER : The determination of molecular formulae of polyols by mass spectrometry of their trimethylsilyl ethers ; the structure of the macrolide antibiotic filipin. Tetrahedron Lett. 1964(37) : 2615~2621, 1964
11) GOLDING, B.T.; R. W. RICKARDS, W. E. MEYER, J. B. PATRICK & M. BARBER : The structure of the macrolide antibiotic pimaricin. Tetrahedron Lett. 1966(47) : 3551~3557, 1966
12) PANDEY, R. C.; V. F. GERMAN, Y. NISHIKAWA & K. L. RINEHART, Jr. : Polyene antibiotics. II. The structure of tetrin A. Submitted.