STRUCTURE AND PROPERTIES OF MAJOR LARGOMYCIN FII CHROMOPHORE COMPONENTS

SYLVIA K. GONDA*, KEVIN M. BYRNE, PATRICIA K. HERBER, YVES TONDEUR, DANIEL LIBERATO† and BRUCE D. HILTON

Program Resources, Inc., National Cancer Institute-Frederick Cancer Research Facility, Frederick, Maryland 21701, U.S.A.
†Laboratory of Theoretical and Physical Biology, NICHD, NIH Bethesda, Maryland 20205, U.S.A.

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Largomycin FII, a protein antitumor antibiotic of molecular weight 29,300 daltons, contains a chromophore that is separable under mild denaturing conditions. The chromophore complex was found to be considerably less stable than the holoprotein towards light and heat, suggesting a protective effect of the protein on the chromophore. Separation of the chromophore into several components was achieved using high performance liquid chromatography, and the biological activity of the isolated components was determined. Data gathered from UV, IR, proton and carbon NMR, and fast atom bombardment mass spectrometry indicated that all the chromophore components belong to the pluramycin class of antitumor agents. Pluramycin A and deacetylpluramycin A were found to be the two major components.

Largomycin FII (LM-FII) is an acidic protein antibiotic isolated from *Streptomyces pluricoloreseens*[1], which exhibits antitumor activity[2]. More than 50 proteins have been reported with antitumor activity[3], several of which (auromomycin[4,5], neocarzinostatin[6], macromomycin[5,7], plurallin[8], and prunacetin A[9]) possess a nonprotein chromophore. Most antitumor chromoproteins range in molecular weight from 9,000 to 13,000 daltons; in contrast, LM-FII has a molecular weight of 29,300[10]. We present data on the isolation, separation, and biological activity of the chromophore complex and apoprotein, and establish the structure of the two major components of the LM-FII chromophore by various spectroscopic techniques. A detailed comparison with other known anthraquinone antitumor agents (pluramycin A[11], hedamycin[12], and kidamycin[13]), is also presented.

Materials and Methods

Highly purified LM-FII was obtained as described previously[10,14].

Isolation of Chromophore

Isolation of LM-FII chromophore was accomplished by adding 100 μl of a 10 mg/ml solution of LM-FII to 1 ml of acetonitrile and 5 μl of formic or glacial acetic acid. The protein precipitates immediately upon addition of the acid, leaving the chromophore in solution. The mixture was vortexed and centrifuged at 5,000 rpm for 10 minutes. The supernatant containing the yellow chromophore was then removed and the pellet was washed twice with 1 ml of acetonitrile. The supernatants were combined and dried under vacuum. As has been previously reported[10,15], extraction with various organic solvents in the absence of acid did not lead to the release of the chromophore.

Biochemical Prophage Induction Assay

The biological activity of the isolated chromophore was investigated using the biochemical prophage induction assay (BIA), which measures a compound's ability to cause DNA damage[16]. *Escherichia coli* strain BR339 containing a *lexA* mutation was used as the BIA host organism. Permeability of this strain was increased by treatment with 1 M Tris-HCl (unpublished data).

HPLC

A Waters Associates model 6000A solvent delivery system equipped with a Schoeffel model SF770 variable-wavelength detector was used. For analytical separations, a Waters Associates μBondapak C_{18} column (30 cm×3.9 mm) was used. The mobile phase consisted of MeOH - H_2O (55: 45) made 100 mM in ammonium acetate and the pH was adjusted to 5.1 with glacial acetic acid. The system was run isocratically at a flow rate of 1 ml/minute. For preparative separations, a Whatman Partisil M9-1050 ODS-3 column (500 mm×9.4 mm) was used. The mobile phase was prepared as above, except that the MeOH - H_2O ratio was changed to 60: 40 and the flow rate was changed to 3 ml/minute. Chromophore components were detected at 254 nm. Separated components were collected from the HPLC in an ice bath, the MeOH was removed under vacuum, and samples were then lyophilized and stored frozen at −20°C.

Antimicrobial Activity

Agars were made 0.01 M with Trizma Base (Sigma, Saint Louis) and adjusted to pH 8.7 with 1 N NaOH before autoclaving. Antibiotic Medium 3 (Difco Laboratories, Detroit) was used for *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*; Mueller-Hinton agar for *Micrococcus luteus*; Sabouraud dextrose agar for *Candida albicans* and *Penicillium notatum*; and Antibiotic Medium 1 (Difco) for *Pseudomonas stutzeri*. Test samples were dissolved in 100 mM ammonium acetate and 15 μl was applied to 6.35 mm discs. The minimal inhibitory concentration of drug was calculated from a standard curve of the zone of inhibition vs. concentration.

Stability Studies

The thermal inactivation of the major HPLC components 7 and 8 was studied using HPLC; biological activity was monitored using the *M. luteus* assay. Stability in water and 100 mM ammonium acetate was determined at ambient temp and 60°C.

Conversion of Chromophore into Free Base Form

Peaks collected from the HPLC were in the salt form. Conversion to the free base form for spectral studies was achieved by dissolving the components in H_2O, adjusting the pH to 8.5 with 1 N NaOH, and extracting three times with $CHCl_3$. The combined chloroform extracts were then dried under reduced pressure and stored at −20°C.

UV Spectroscopy

The spectra of chromophore components dissolved in H_2O, pH 7.0, at a concentration equivalent to 5 mg/ml of extracted LM-FII were recorded on a Perkin-Elmer Lambda 5 spectrophotometer.

IR Spectrometry

Samples were dissolved in MeOH or $CHCl_3$ and dried under a stream of N_2 on a KBr salt pellet. Spectra were obtained on a Perkin-Elmer 180 spectrophotometer.

Fast Atom Bombardment Mass Spectrometry

Fast atom bombardment mass spectra (FAB-MS) were obtained using a ZAB-2F (VG Analytical, Altrincham, UK) interfaced to a VG 2035 data system. Xenon atoms (8 KeV, 1 mA plasma discharge current; Ion Tech Ltd. gun, Middlesex, UK) were used as the ionizing particles. A mixture of glycerol-*N*,*N*-dimethylformamide (1: 1) was used as the supporting matrix. The scanning rate was 10 seconds/decade with a resolution of 1,000. A typical spectrum of glycerol was saved in the computer memory and subsequently used for "background" subtraction. High resolution peak matching measurements were performed at 10,000 resolution (5% crossover definition) using $[Na_4I_5]$ (*m*/*z* 726.48142).

NMR Spectroscopy

Spectra were obtained utilizing 2~5 mg samples dissolved in 0.5 ml $CDCl_3$ (Merck) in a 5-mm tube. A Nicolet (GE Medical System) NT-300 spectrometer with 1280 data system was used in conjunction with tuned 5-mm carbon (75.46 MHz) and proton probes (300.04 MHz). Carbon spectra were broadband decoupled using Nicolet's implementation of Levitt (MLEV 16) decoupling. Homonuclear two-dimensional (2-D) proton correlation spectra were obtained using Nicolet's COSY sequence with 512 ×1K data points accumulated, sine bell apodized in both dimensions to give a 512×512 transformed data block. Thirty-six minutes was required for the 2-D acquisitions; 48 hours was required for the carbon spectra. Proton spectra were reproducible over time periods in excess of one month.

Results

Biological Properties

Using the spot test version of the BIA[17] and the permeabilized BR339 strain, LM-FII was detected at a concentration of 0.30 μg/ml, chromophore complex was detected at a concentration equivalent to 0.60 μg/ml of extracted LM-FII, and the apoprotein showed no biological activity at 1,000 μg/ml.

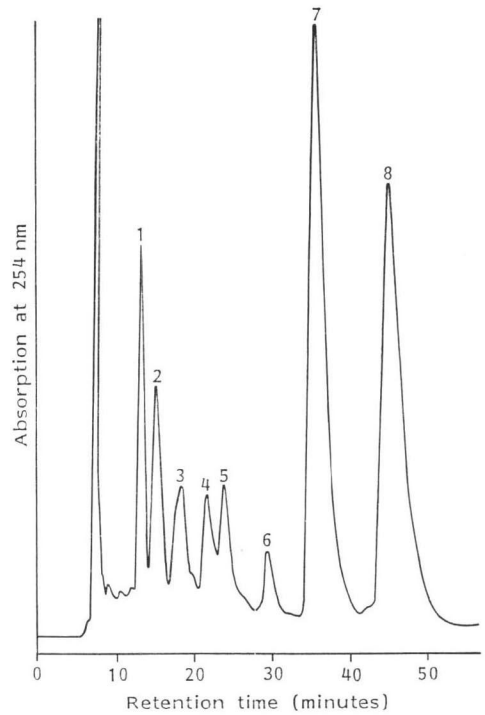
HPLC Analysis

An HPLC system was developed to examine the chromophore. The basic character of the chromophore necessitated the use of ion-pairing chromatography. The complex profile of the chromophore extract is shown in Fig. 1. Components corresponding to peaks 7 and 8 were present in the greatest abundance and possessed a majority of the *in vitro* biological activity. Reinjection of components 7 and 8 into the HPLC led to single peaks, ruling out the possibility that the material was undergoing decomposition on HPLC.

When the major peaks were collected, evaporated to dryness, and reexposed to the identical conditions used to initially extract the chromophore, no evidence of decomposition was apparent by HPLC analysis.

Fig. 1. HPLC profile of LM-FII chromophore complex (2.0 mg equivalent weight of LM-FII dissolved in 0.1 mM phosphate buffer, pH 7.0) using reverse-phase paired-ion chromatography.

Chromatography was carried out as described under Materials and Methods for analytical separations (0.1 Aufs).



Antimicrobial Spectrum

Individual components separated by HPLC were collected, and after removal of the methanol, samples were lyophilized. Each component was then dissolved in 100 mM ammonium acetate and a

Table 1. Antimicrobial spectrum of chromophore components.

Test organism	MIC of HPLC components[a] (μg/ml)[b]							
	1	2	3	4	5	6	7	8
Staphylococcus aureus ATCC 6538P	50	50	25	5	25	25	5	5
Bacillus subtilis ATCC 6633	25	25	5	0.5	2.5	2.5	1.0	2.5
Micrococcus luteus ATCC 9341	5	2.5	2.5	0.1	1.0	1.0	0.5	0.5
Escherichia coli ATCC 10536	>100	>100	>100	25	100	100	25	50
Pseudomonas stutzeri ATCC 11607	100	100	50	25	25	50	25	50
Candida albicans ATCC 10231	>100	>100	>100	>100	>100	>100	>100	>100
Penicillium notatum ATCC 9478	>100	>100	>100	100	>100	>100	25	50

[a] Components assigned from HPLC profile shown in Fig. 1.

[b] Concentrations were calculated from optical density measurements taken at 430 nm with an $E^{1\%}_{1cm}$ value of 87, determined from an authentic sample of hedamycin in the same solvent.

Fig. 2. Stability of LM-FII and its chromophore to UV treatment.

UV irradiation was carried out at a distance of 15 cm from UV_A lamp (Sylvania lifeline FR40T12) for the indicated time. The biological activity is reported as a percentage of the initial activity calculated in units of β-galactosidase.[17]

○ 10 μg/ml LM-FII, ● 20 μg/ml LM-FII chromophore.

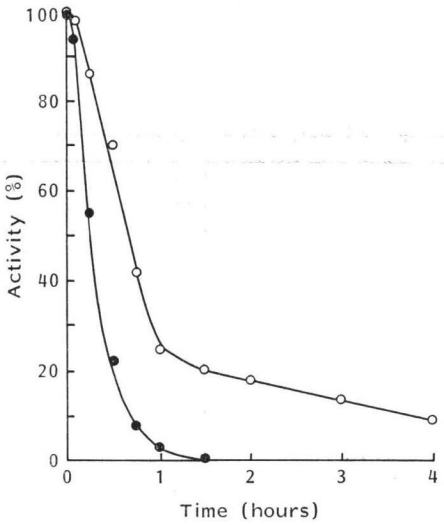
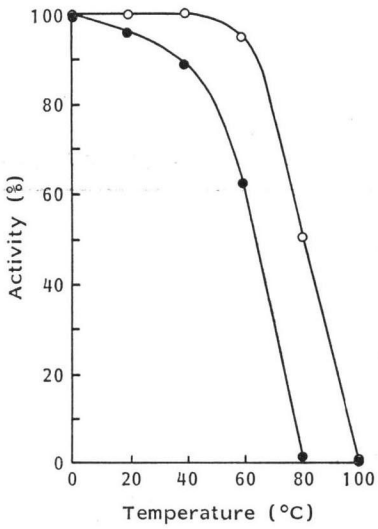


Fig. 3. Stability of LM-FII and its chromophore to heat treatment.

Samples were incubated for 2 hours at the indicated temperature in the dark. The biological activity is reported as a percentage of the initial activity calculated in units of β-galactosidase[17].

○ 10 μg/ml LM-FII, ● 20 μg/ml LM-FII chromophore.



small aliquot of each was reinjected on the HPLC to check its homogeneity. Antimicrobial activity against a number of organisms showed that all peaks possess biological activity (Table 1). Components 4, 7, and 8 possessed the highest level of activity, with component 4 showing better activity against Gram-positive bacteria, while components 7 and 8 displayed higher activity against the fungus *P. notatum*. Efforts to isolate and characterize component 4 are currently in progress.

Stability Studies

Inactivation of the chromophore by light and heat was examined using HPLC and BIA. When 1 mg equivalent of LM-FII was dissolved in 1 ml of 100 mM ammonium acetate and exposed for 1 hour to a UV_A lamp, all biological activity was lost. Accompanying the loss of the biological activity was a reduction in all HPLC peak heights, along with a color change in the solution from yellow to orange.

Light inactivation of the chromophore and LM-FII was also compared using the quantitative BIA. The results are shown in Fig. 2. After half an hour of UV exposure, the chromophore lost 80% of its activity and all of its activity after 1.5 hours. The LM-FII, however, lost 25% of its activity after half an hour and still had 10% of its activity at the end of 4 hours of exposure to UV light. Fig. 3 shows the thermal inactivation of the chromophore and LM-FII. After 2 hours at 60°C, the chromophore lost 40% of its activity, while LM-FII lost less than 5% of its activity. In both types of inactivation, intact LM-FII showed greater stability than the free chromophore.

The stability of components 7 and 8 was also examined qualitatively. When component 7 was heated in water or in 100 mM ammonium acetate and then assayed by HPLC, several components with earlier retention times (more polar) appeared. When component 8 was heated in water, it also decom-

Fig. 4. FAB-MS of component 7 from the LM-FII chromophore complex.
(A) Positive ion, (B) Negative ion.

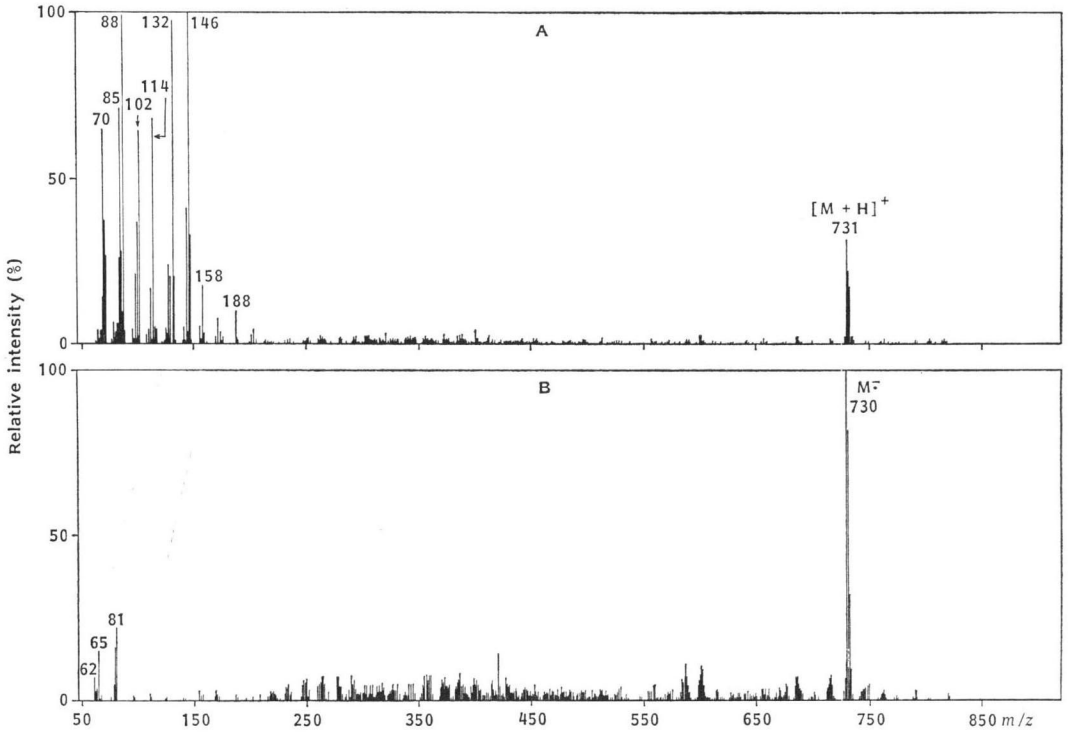
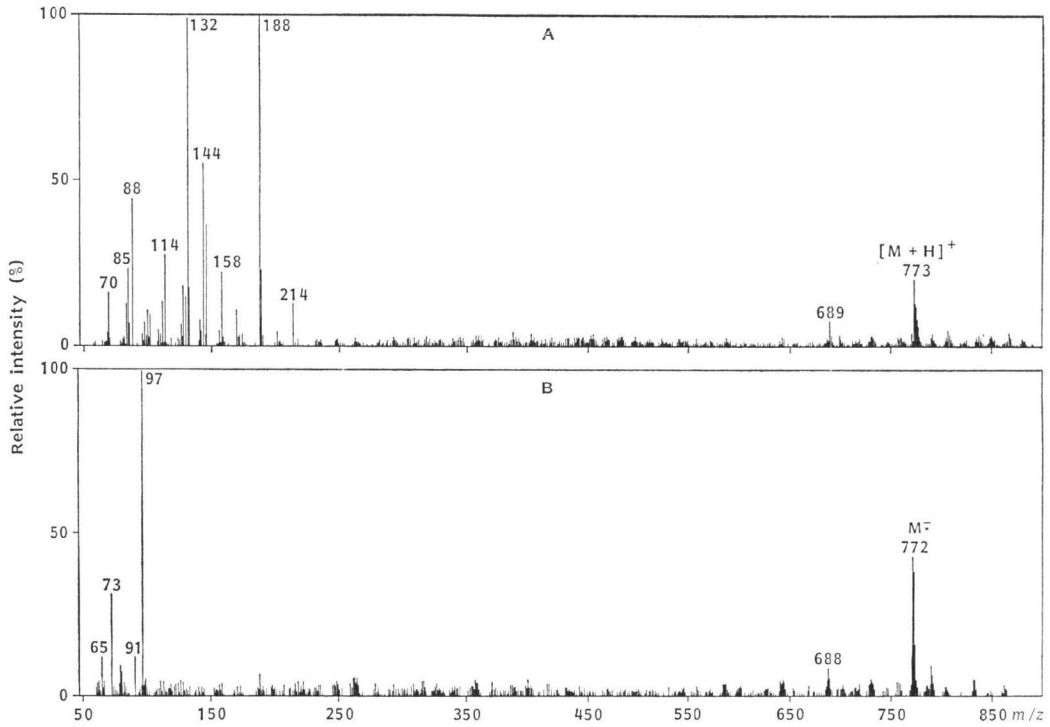
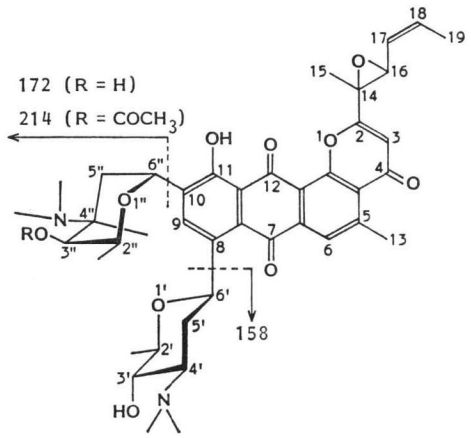


Fig. 5. FAB-MS of component 8 from the LM-FII chromophore complex.
(A) Positive ion, (B) Negative ion.



posed into several components with earlier retention times. However, when component 8 was heated in 100 mM ammonium acetate, most of it was converted to component 7 before decomposing into more polar products. Biological activity was maintained for components 7 and 8, but there was diminished activity associated with decomposition products with earlier retention times.

Fig. 6. Structure of components 7 and 8 with major fragmentations.



Deacetylpluramycin A (component 7) R=H
Pluramycin A (component 8) R=$COCH_3$

Spectrometry

UV spectra of all the components were very similar. All had UV maxima of 243 nm, 269 nm, and 425 nm in water at pH 7.0.

IR spectra were also very similar for the different components. Component 7 showed bands at the following wave numbers in the IR spectrum: 3450, 2980, 2940, 2880, 2795, 1655, 1620, 1580, 1460, 1435, 1430, 1420, 1365, 1300, 1250, 1220, 1170, 1150, 1075, 1040, 990, 960, 895, 835, 780, 760, and 680 cm^{-1}. Component 8 had the same bands as component 7, with an additional peak at 1730, which is indicative of a carbonyl stretching frequency of an acetate group. This IR spectrum was identical to the published spectrum of pluramycin A[11].

The recently developed ionization technique of FAB-MS[18] has been applied to the chromophore complex. The positive and negative ion FAB spectra from the major components labeled 7 and 8 are shown in Figs. 4 and 5. The FAB spectra of component 7 (Fig. 4) are characterized by a pseudomolecular ion $[M+H]^+$ (*m/z* 731) and a molecular anion $[M]^-$ (*m/z* 730). The elemental composition of the molecular ion cluster in the positive ion FAB-MS (Fig. 4A) was determined as $C_{41}H_{51}N_2O_{10}$ (731.3571, *i.e.*, −3.5 ppm) using peak matching. Similarly the FAB spectra of component 8 (Fig. 5) and pluramycin A are characterized by an abundant pseudomolecular ion $[M+H]^+$ (*m/z* 773) and molecular anion $[M]^-$ (*m/z* 772), as expected for pluramycin A and determined from an authentic sample. The

Fig. 7. Relationships between the various components of the chromophore complex as determined by mass spectrometry.

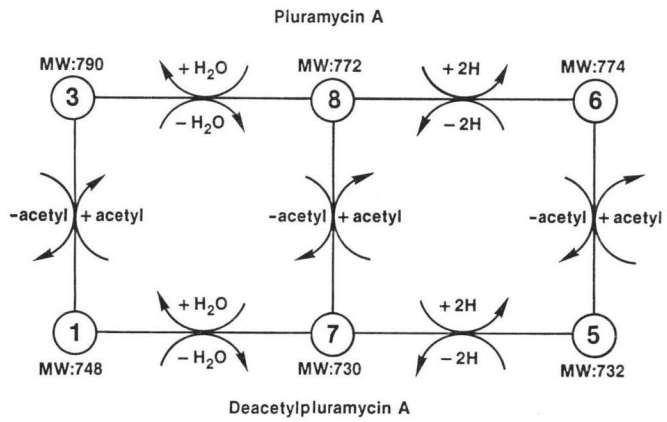


Table 2. NMR values for LM-FII chromophore components 7 and 8[a].

	^{13}C NMR			^{1}H NMR		
	7	8	Ref	7	8	Ref
2	167.3	167.3	166.3[b]	—	—	—
3	109.8	109.9	110.0[b]	6.50	6.50	6.50[e]
4	178.8	178.8	178.7[b]	—	—	—
4a	126.1*	126.0	125.8*[b]	—	—	—
5	149.7	149.7	149.7[b]	—	—	—
6	125.7	125.7	125.9[b]	8.00	8.00	7.99[e]
6a	137.1	137.2	137.3[b]	—	—	—
7	183.1	183.2	183.1[b]	—	—	—
7a	126.1*	126.1*	126.2*[b]	—	—	—
8	140.0	140.2	140.2[b]	—	—	—
9	132.9	134.0	133.1[b]	8.30	8.30	8.27s[e]
10	138.2	138.2	138.6[b]	—	—	—
11	159.7	159.3	159.3[b]	—	—	—
11a	115.9	115.7	116.1[b]	—	—	—
12	187.9	187.7	188.0[b]	—	—	—
12a	119.1	119.1	119.2[b]	—	—	—
12b	155.9	155.9	156.1[b]	—	—	—
13	24.1	24.1	24.1[b]	3.00	3.00	3.00[e]
14	59.0	59.0	60.3[c]	—	—	—
15	14.3	13.8	14.9[c]	1.80	1.85	1.83[e]
16	61.5	61.7	61.7[c]	4.15d	4.14d	4.15d[e]
17	123.0	123.1	123.3[c]	5.40m	5.40m	5.4m[e]
18	134.0	132.4	134.1[c]	6.05dq	6.05dq	6.05m[e]
19	14.0	14.3	14.4[c]	1.90dd	1.90dd	1.88dd[e]
11-OH	—	—	—	14.05	13.90	13.92[e]
2′	77.1	77.5	77.3[b]	3.60m	3.50dq	3.56m[e]
3′	71.6	71.4	71.9[b]	3.25t	3.30t	3.26t[e]
4′	67.3	67.6	67.4[b]	2.95m	2.95m	2.87m[e]
5′	28.2	28.7	28.3[b]	ax 1.41* eq 2.31*	ax 1.45* eq 2.21*	ax 1.39m[e] eq ~1.6[e]
6′	74.9	75.1	75.2[b]	5.45	5.40m	5.43d[e]
2′-CH_3	18.8	18.8	18.9[b]	1.45d	1.45	1.46d[e]
4′-$N(CH_3)_2$	40.3	40.3	40.4[b]	2.25s	2.40	2.33s[e]
2″	66.9	69.8	69.9[d] 67.3[b]	4.05	4.30dq	4.32m[e]
3″	70.6	65.0br	64.8[d] 70.9[b]	3.40	5.15d	5.22d[e]
4″	57.3	57.5br	57.7[d] 57.3[b]	—	—	—
5″	33.1	40.6br	41.1[d] 33.7[b]	ax2.60m eq2.30m	2.50m	NR
6″	69.6	77.1	76.3[d] 69.6[b]	5.50m	5.55dd	5.38m[e]
2″-CH_3	17.6	14.8br	15.0[d] 17.6[b]	1.50d	1.50d	1.44d[e]
4″-CH_3	12.2	13.7br	13.7[d] 12.3[b]	0.75	0.95	0.99[e]
4″-$N(CH_3)_2$	36.6	39.2br	39.4[d] 36.8[b]	2.30	2.40	2.29[e]
3″-OCO–	—	170.5br	170.4[d]	—	—	—
3″-$OCOCH_3$	—	21.2br	21.2[d]	—	2.20	2.19[e]

[a] All numerical values are chemical shifts in ppm vs. internal TMS. Proton values are ±0.05; carbon values are ±0.1.
*=assignments may be interchanged.
dq=doublet of quartets. br=broad. NR=not resolved. ax=axial, eq=equatorial.

[b] Values for hedamycin[21].

[c] Values for pluramycin A[23].

[d] Values for kidamycin-3′,3″-diacetate[21].

[e] Values for pluramycin A[20].

Table 3. Proton-proton coupling constants and two-dimensional connectivities for LM-FII chromophore components 7 and 8.

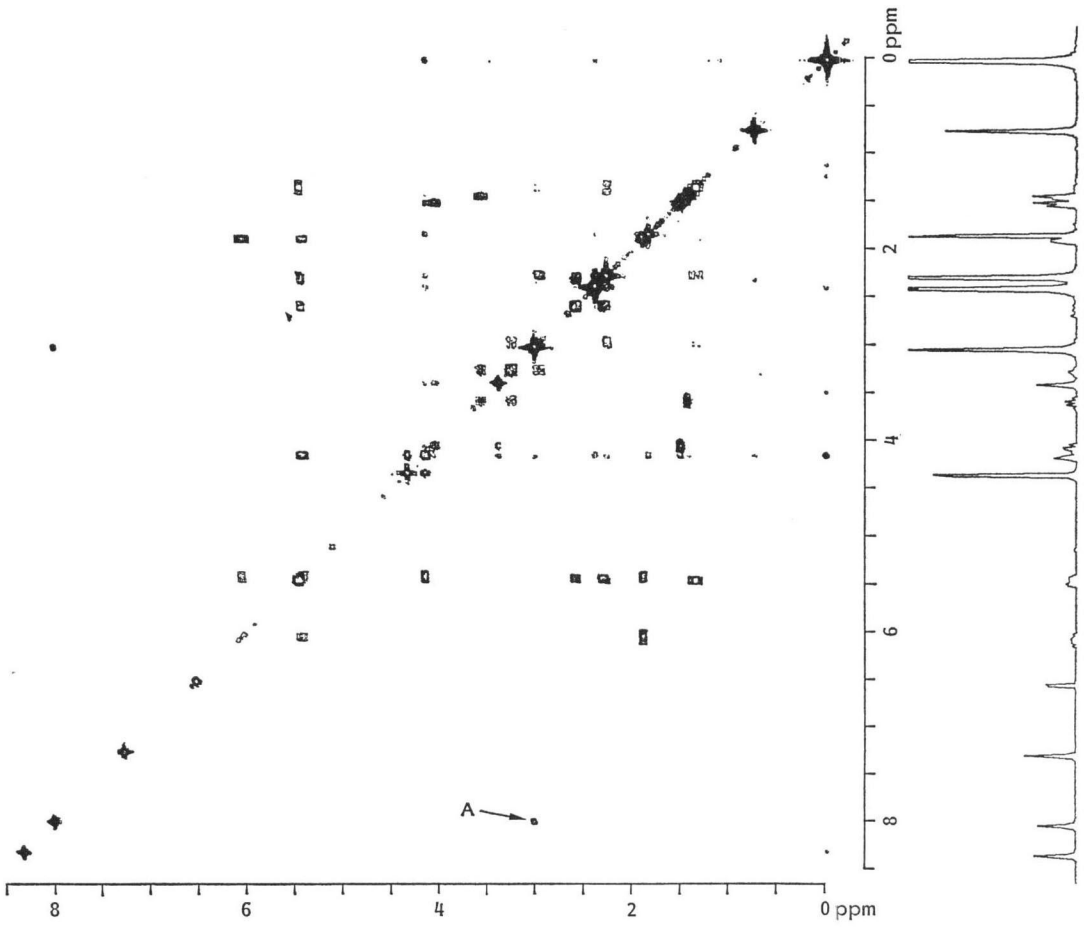
Connection	7		8	
	J (Hz)	2D connectivity	*J* (Hz)	2D connectivity
2′, 3′	8.5	+	8.6	+
2′, 7′	6.0	+	6.1	+
3′, 4′	9.2	+	9.2	+
2″, 3″	<2.0	+	4.5	+
2″, 7″	6.5	+	6.7	+
5′ax, 5′eq	10.1	+	12.0	+
5″ax, 5″eq	13.9	+	—	+
5″ax, 6″	3.9	+	3.6	+
16, 17	7.8	+	7.7	+
17, 18	11.2	+	11.2	+
17, 19	1.5	+	1.8	+
18, 19	7.1	+	7.1	+
18, 16	—	—	0.9	—
6′, 5′ax	—	+	1.8	+
6′, 5′eq	—	—	9.8	+
6″, 5″eq	—	+	8.8	+
4′, 5′ax	—	—	3.6	+
4′, 5′eq	—	+	9.0	+
9, 6″	—	—	—	+
6, 13	—	+	—	+

+ Indicates that a cross peak was observed in the homonuclear correlation spectrum. Coupling constants are estimated to be ± 0.2 Hz.

elemental compositions of the two major peaks in the molecular ion cluster (Fig. 5B) were determined as $C_{43}H_{52}N_2O_{11}$ (772.3551, *i.e.*, +2.6 ppm for component 8, and 772.3602, *i.e.*, −4 ppm for the standard compound) and $C_{43}H_{53}N_2O_{11}$ (773.3620, *i.e.*, +3.8 ppm) using peak matching. Fragment ions (Figs. 5 and 6) characteristic of the two sugar rings (*m/z* 158, *m/z* 214) are detected in the positive ion FAB spectrum of component 8. Similarly, fragment ions characteristic of the two sugar rings *m/z* 158 and *m/z* 172 for the deacetylated sugar ring are present in the positive ion FAB spectrum of component 7 (Fig. 4A). The base peak of *m/z* 188, originates from the probable loss of C_2H_2 from the acetylated sugar ring, and the intense signal obtained at *m/z* 132 results from the loss of C_2H_2 from the nonacetylated sugar moiety (*m/z* 158) of component 8. Similar losses are observed for component 7 at *m/z* 146 and 132. Most of the signals at lower masses in the positive ion FAB spectra of components 7 and 8 are probably due to sugar fragmentations. The signal at *m/z* 688 has been identified as a minor impurity in component 8. This impurity was isolated using a different C-18 reverse phase HPLC column and has the same MW and apparent HPLC retention time as kidamycin which is another pluramycin-type antibiotic (unpublished results). Fig. 7 summarizes the MW findings for the various components of the chromophoric mixture and their relationship based on mass spectrometric determinations.

The NMR spectra indicate that the purity of components 7 and 8 was greater than 95%. The carbon chemical shifts, proton chemical shifts, proton multiplet appearance, proton-proton coupling constants, and proton-proton 2-D NMR connectivities are shown in Tables 2 and 3. Chemical shifts cited in Table 2 are assigned using the appropriate analogous subgroups of several similar known model compounds[19~23]. The subgroups of the parent structure chosen (Fig. 6) are the two sugar rings, the quinoidal system (C2~C12), and the epoxypropenyl side chain (C14~C19). Atoms at positions where

Fig. 8. Two-dimensional NMR spectrum of component 7 from the LM-FII chromophore complex.



the subgroups join together have chemical shifts that vary predictably from those reported in the literature in situations where data for the appropriate model parent compound are not available. For example, carbon 2 in either fraction has a chemical shift 1.0 ppm downfield from that cited in the literature, because this carbon is the site for attachment of the pluramycin side chain.

Data for the absolute configuration of these compounds have not been firmly established. The assignments of the methylene protons in the sugar rings to axial or equatorial configuration are speculative. The C-17, C-18 double bond is *cis*, in agreement with previous work[23], based on the magnitude of the H-17, H-18 coupling constant and on the agreement between the measured carbon chemical shifts and the chemical shifts of model compounds[23].

An interesting phenomenon that served to verify assignments is the broadness of the carbon resonances of the sugar ring (C2″ ~ C6″) in component 8. It is suggested that this reflects slow configurational rearrangement of this ring on the NMR time scale.

The assignment and confirmation of the primary structure of the compounds from component 7 and from component 8 were greatly facilitated by use of 2-D NMR. Fig. 8 shows the 2-D spectrum of component 7. In this homonuclear correlation plot, the one-dimension spectrum lies along the diagonal and is projected on the right axis. Off-diagonal cross peaks or connectivities indicate the presence of

scalar (J) coupling (Table 3). Thus, the cross peak at A in the figure shows a long-range coupling between the proton at position 6 (8.0 ppm) and the methyl at position 13 (3.0 ppm). The 2-D spectra were indispensable for determination of chemical shifts and assignments of several sugar ring protons that are obscured in the one-dimensional spectrum by the resonances of methyl or dimethyl groups.

The FAB-MS showed a difference of MW of 42 between components 7 and 8 which is attributable to an acetyl group. The presence of an acetyl group is evident in the IR spectrum of component 8, where there is an extra band at 1730 cm^{-1} due to the carbonyl stretching frequency of an acetate group and is also present in the ^{1}H and ^{13}C NMR where there are 2 extra carbons and 3 extra protons on the C-3″ position of component 8, not present in the spectra of component 7. Based on the UV, IR, ^{1}H and ^{13}C NMR and FAB-MS data, deacetylpluramycin A and pluramycin A are assigned to components 7 and 8 respectively.

Discussion

LM-FII is a chromoprotein that exhibits antitumor activity[2]. The data indicate that all the biological activity is associated with the chromophore, as is the case with the chromoproteins neocarzinostatin[24], auromomycin, and macromomycin[25].

Using reverse-phase ion-pairing chromatography, we were able to separate the LM-FII chromophore into a number of components. These components seem to be present in the fermentation broths at different concentrations. Ratios of different components have varied from fermentation to fermentation, but components 7 and 8 were always present as major products; therefore, they were studied in some detail. Determination of the molecular weights was made possible using FAB-MS, which is ideally suited for high molecular weight, polar, and thermally labile compounds. In fact, there are no published mass spectra on these compounds because of their instability. Spectra were obtained equally well for positive and negative ions (Figs. 4 and 5). FAB spectra of component 8 and pluramycin A showed the same molecular ion and fragmentation patterns. By straightforward comparisons with existing data and 2-D NMR the primary structure of the compounds as pluramycin A for component 8 and deacetylpluramycin A for component 7 was firmly established.

As reported for neocarzinostatin[24], macromomycin, and auromomycin[25], exposure of the chromophore and LM-FII to UV light (Fig. 2) demonstrated the lability of the free chromophore in aqueous solution and the stabilizing effect of association with its apoprotein. The chemical transformations that take place upon irradiation of a number of different epoxide side chains on the pluramycin class of antibiotics were recently investigated[26]. It was found that ketones and alcohols, as well as decarboxylation products, were formed upon exposure to light. It was concluded that the photochemical alteration of the side chain would not be expected to greatly affect the biological activity, since quite a number of highly active compounds of the pluramycin type are known which differ in the structure of the side chain. This holds true in our system as well, where components 1, 2, 3, 4, 5, and 6 are believed to differ from 7 and 8 in the side chain based on mass spectrometry, yet all have biological activity associated with them. This implies that since biological activity is lost upon exposure to light, more dramatic changes must be taking place in other parts of the molecule.

The LM-FII chromophore also exhibited lability to heat treatment (Fig. 3). However, it appears to be considerably more stable than the isolated chromophores of neocarzinostatin[27], macromomycin, and auromomycin[25]. LM-FII chromophore lost 12% of its activity after 2 hours at 40°C and 37% at 60°C. Macromomycin chromophore is reported to lose 80% of its activity after 30 minutes at 40°C and all of its activity at 60°C, while neocarzinostatin loses 80% of its activity after 10 minutes at 37°C. In either type of inactivation (light or heat), the apoprotein, though not necessary for activity, does provide stability to the chromophore.

Pluramycin A was discovered as a free chromophore[11] although it was noticed that the chromophore could be stabilized by addition of serum. Plurallin[8] was reported to be a glycoprotein with a

pluramycin-like prosthetic group. It is produced by *Streptomyces pluricolorescens*, the same organism that produces LM-FII, and its molecular weight was estimated by Sephadex G-75 gel permeation chromatography to be 30,000~60,000 daltons (LM-FII is 29,300 daltons). At the time of its discovery, LM-FII[15] was compared with plurallin and found to differ in several ways. Only plurallin gave a positive Elson-Morgan test; after acid hydrolysis, plurallin yielded cysteine, which was not obtained from LM-FII. When the pH indicator chromophore was extracted from LM-FII by the same method as used with plurallin, it had a different IR spectrum from the indicator prosthetic group of plurallin. Careful examination of the data presented in these two papers[8,15] leads us to believe that the IR spectrum published for the chromophore in the latter paper is presumably due to component 7 (deacetylpluramycin A), while the IR spectrum of the plurallin prosthetic group, which differs from the reported LM-FII pigment in that it contains an extra band at 1740, is due to component 8 (pluramycin A). In fact, we have found that all of component 8 eventually converts to the deacetylated form (component 7) during the fermentation process.

The *in vitro* experiments demonstrate that the chromophore possesses most, if not all, of the biological activity. Studies on the mechanism of action of pluramycin A[28~30], neopluramycin[31,32], acetylkidamycin[33~35], and hedamycin[36~40] have shown that these compounds bind very tightly to DNA and inhibit DNA, RNA, and protein syntheses. It is postulated that because of their planar structure and their ability to cause conformational changes in the DNA, binding may involve intercalation into DNA.

In addition to stabilizing the chromophore, the apoprotein may also play a role in lowering toxicity. In fact, pluramycin A, when bound to human serum albumin[41], was found to be less toxic than pluramycin A and showed better antitumor activity; the therapeutic index was more than 10 times higher than pluramycin A itself. *In vivo*, the apoprotein may aid in transporting the chromophore to its target and may play a role in regulating the availability of the chromophore for interaction with DNA.

Addendum in Proof

FAB-MS molecular weight data was also confirmed by thermospray LC-MS.

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