

PHYSICOCHEMICAL AND BIOLOGICAL COMPARISON OF POLYENE
MACROLIDE ANTIBIOTICS FUNGICHROMIN,
LAGOSIN AND COGOMYCIN

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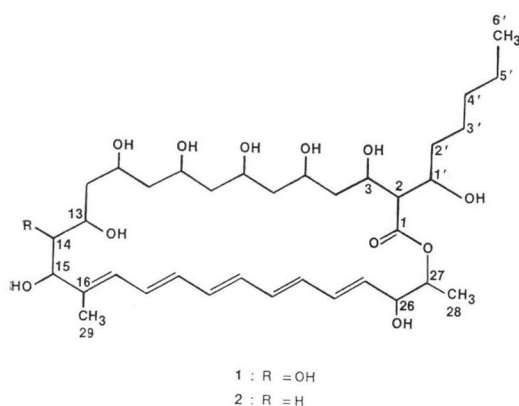
The three polyene macrolide antibiotics, fungichromin, lagosin, and cogomycin, previously described as having some stereochemical differences at one or more centers, are shown by countercurrent distribution, high-performance liquid chromatography, carbon-13 nuclear magnetic resonance spectroscopy, circular dichroism, and biological studies to be identical in all respects, including stereochemical aspects. The differences observed earlier in their properties have now been ascribed to varying amounts of impurities, which are separable by high-performance liquid chromatography. All three antibiotics contain one major and several minor components.

Structure **1** has been assigned to fungichromin^{1,2)}, lagosin^{3,4)}, and cogomycin^{5,6)}. Because these compounds showed some physicochemical differences (Table 1), all of the above names were retained, and it was suggested that there could be stereochemical differences at one or more asymmetric centers. No direct comparison of significant value has ever been carried out to prove the identity of these antibiotics.

While looking for new antitumor compounds of microbial origin, we isolated a polyene macrolide antibiotic from *Streptomyces griseus* (FCRC-21), which was identified subsequently⁷⁾ as fungichromin

Table 1. Physicochemical properties of fungichromin, lagosin, and cogomycin.

Property	Fungichromin (NSC-277813)		Lagosin	Cogomycin
Producing organism	<i>Streptomyces cellulosa</i> ^{1,2)}	<i>S. griseus</i> (FCRC-21) ⁷⁾	<i>S. roseoluteus</i> ^{3,4)}	<i>S. fradiae</i> ^{5,6)}
Nature	Light yellow crystals	Light yellow crystals	Light yellow crystals	Light yellow crystals
Molecular formula	C ₃₅ H ₅₅ O ₁₂	C ₃₅ H ₅₅ O ₁₂	C ₃₅ H ₅₅ O ₁₂	C ₃₅ H ₅₅ O ₁₂
Molecular weight	670	670	670	670
Melting point (°C)	205~210	220~225	230~240	190~210
[α] _D	-176±4° (MeOH)	-227.7° (DMF)	-160±4° (MeOH) -279±3° (DMF)	-157° (MeOH)
IR (cm ⁻¹)	3268, 1709, 1639, 1138, 1010, 846	3440, 1725, 1635, 1135, 1005, 850	3310, 1710, 1635, 1136, 1010, 852	1730, 1005
UV λ _{max} (E _{1cm} ^{1%})	357(1460), 339(1550), 323(960), 310(sh)	357(1231), 338(1250), 322(786), 308(sh)	356(1480), 338(1490), 322(930), 308(420)	357(1270), 339(1300), 322(731), 309(326)



and assigned structure **1**. During the course of this work it was possible to obtain samples of fungichromin, lagosin, and cogomycin and carry out a detailed, direct comparison using counter-current distribution (CCD), high-performance liquid chromatography (HPLC), carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR), circular dichroism (CD), and examine some biological properties. In this paper these findings are discussed and we suggest that all these antibiotics are the same. Furthermore, because fungichromin was the first of these antibiotics to be reported in the literature its name deserves precedence and the use of the synonyms lagosin and cogomycin should be discontinued.

Materials and Methods

Fungichromin

(a) An authentic sample of fungichromin was obtained from Merck Institute for Therapeutic Research, Rahway, New Jersey. (b) A sample isolated in our laboratory and identified as fungichromin⁷⁾ was also used.

Lagosin

Authentic samples of lagosin were obtained from Dyson Perrins Laboratory, England and Glaxo Research Ltd., England. These samples were purified by CCD before comparison with fungichromin and cogomycin.

Cogomycin

A sample of cogomycin was supplied by Dr. V. POZSGAY of the Institute of Biochemistry, L. Kossuth University, Debrecen, Hungary. This material was also purified by CCD before use.

CCD of Fungichromin, Lagosin and Cogomycin

A 500-tube CCD apparatus (H. O. Post Scientific Instrument Co. Inc., N. Y.) was used. Purified fungichromin, lagosin, and cogomycin were loaded in two tubes each, 165 tubes apart in the solvent system chloroform - methanol - borate buffer (pH 8.3, 0.05 M), 2: 2: 1. Five hundred transfers were performed in such a way that at no stage did any one antibiotic overlap another. The movement was followed by measuring optical density at 357 nm in an ultraviolet-visible (UV-Vis) spectrophotometer and plotting a CCD curve for each of the antibiotics. Based on the distribution coefficients (K), the theoretical curves were calculated. A comparison of CCD curves is shown in Fig. 1.

HPLC of Fungichromin, Lagosin and Cogomycin

HPLC was performed using a Waters Associates M-6000A pump with a Model U6K injector. The analytical separations were performed on a Waters C_{18} μ Bondapak reverse phase column (3.9 mm ID \times 30 cm) and were monitored by a Schoeffel Spectroflow SF 770 variable UV detector or ISCO UA-5 UV detector and an Omniscribe recorder. A solvent system composed of methanol - water (60: 40) was used for all of the separations.

Thin-Layer Chromatography (TLC)

TLC was performed on precoated TLC plates (silica gel GF, 250 μ Analtech). UV light, iodine, or sulfuric acid spray was used to visualize the spots.

^{13}C NMR

^{13}C NMR spectra were taken on a JEOL-FX60 spectrometer. The spectra were measured in 10-

mm sample tubes, employing pyridine- d_5 as the solvent, tetramethyl silane ($(\text{CH}_3)_4\text{Si}$) as internal standard and an internal deuterium lock. Chemical shifts are reported in parts per million (ppm) from $(\text{CH}_3)_4\text{Si}$ as internal standard.

General Instrumentation

Melting points were determined on a Kofler micro hot stage apparatus and are uncorrected. UV-Vis spectra were taken in methanol on a GCA/McPherson Model EU-700-32 spectrophotometer, infrared (IR) spectra were recorded on a Beckman IR-12 spectrophotometer, and CD spectra were recorded on a Jasco Model J-40 automatic recording spectropolarimeter.

Biological Studies

The methods reported earlier⁹⁾ were used to measure the amount of potassium and hemoglobin remaining in the cells and the viability of yeast cells.

Studies of *in vivo* activity against P388 murine tumor cell lines were performed according to published National Cancer Institute protocols⁹⁾.

Results

A comparison of CCD studies of fungichromin, lagosin, and cogomycin under identical conditions, using chloroform - methanol - borate buffer (pH 8.3, 0.05 M), 2: 2: 1, is shown in Fig. 1 and Table 2. All of them have identical distribution coefficients(K), the difference of ± 0.033 being within the experimental error.

The HPLC comparison on a C_{18} μ Bondapak reverse phase column using methanol - water (60: 40) as the solvent system (Fig. 2) showed that all the antibiotics discussed here have one major component (peak #6, RT=6 minutes, 74~86%) and several minor components. One of the minor components (peak #7, RT=7.5 minutes, 6~10%) was identified as filipin III (2), the major component of the filipin complex^{10,11)}, by HPLC comparison. As expected, the filipin complex showed, in addition to various filipins, fungichromin as one of the minor components.

TLC comparison of fungichromin, lagosin, and cogomycin using two solvent systems showed that they are indistinguishable (Table 2).

Table 2. Properties of countercurrent distribution (CCD) purified fungichromin, lagosin, cogomycin, and filipin III.

	Melting point ($^{\circ}\text{C}$)	TLC* ² (Rf)		CCD* ³	HPLC* ⁴
		Solvent			
		A	B		
Fungichromin* ¹	157~162	0.27	0.67	1.35 ^a 2.57 ^b	6.00
Lagosin	158~163	0.28	0.69	1.29 ^a 2.57 ^b	6.00
Cogomycin	157~165	0.28	0.68	1.33 ^a 2.61 ^b	6.00
Filipin III	119~128	0.45	0.81	1.45 ^b	7.50

*¹ Product obtained at NCI-Frederick Cancer Research Facility from *S. griseus* (FCRC-21).⁷⁾

*² Solvent A: CHCl_3 - MeOH (85: 15). Solvent B: *n*-BuOH - CH_3COOH - H_2O (4: 1: 5, upper layer).

*³ K at 23 $^{\circ}\text{C}$. a: CHCl_3 - MeOH - borate buffer (0.05 M, pH 8.3), 2: 2: 1. b: CHCl_3 - MeOH - borate buffer (0.05 M, pH 9.2), 2: 2: 1.

*⁴ RT of major peak in minutes. C_{18} μ Bondapak column (3.9 mm \times 30 cm), solvent: MeOH - H_2O (60: 40), flow: 1.5 ml/minute.

Fig. 1. Comparison of countercurrent distribution studies of fungichromin (top), lagosin (center), and cogomycin (bottom) using chloroform - methanol - borate buffer (pH 8.3, 0.05 M), 2: 2: 1 as the solvent system. Total number of transfers (n), 500; temperature, 23°C.

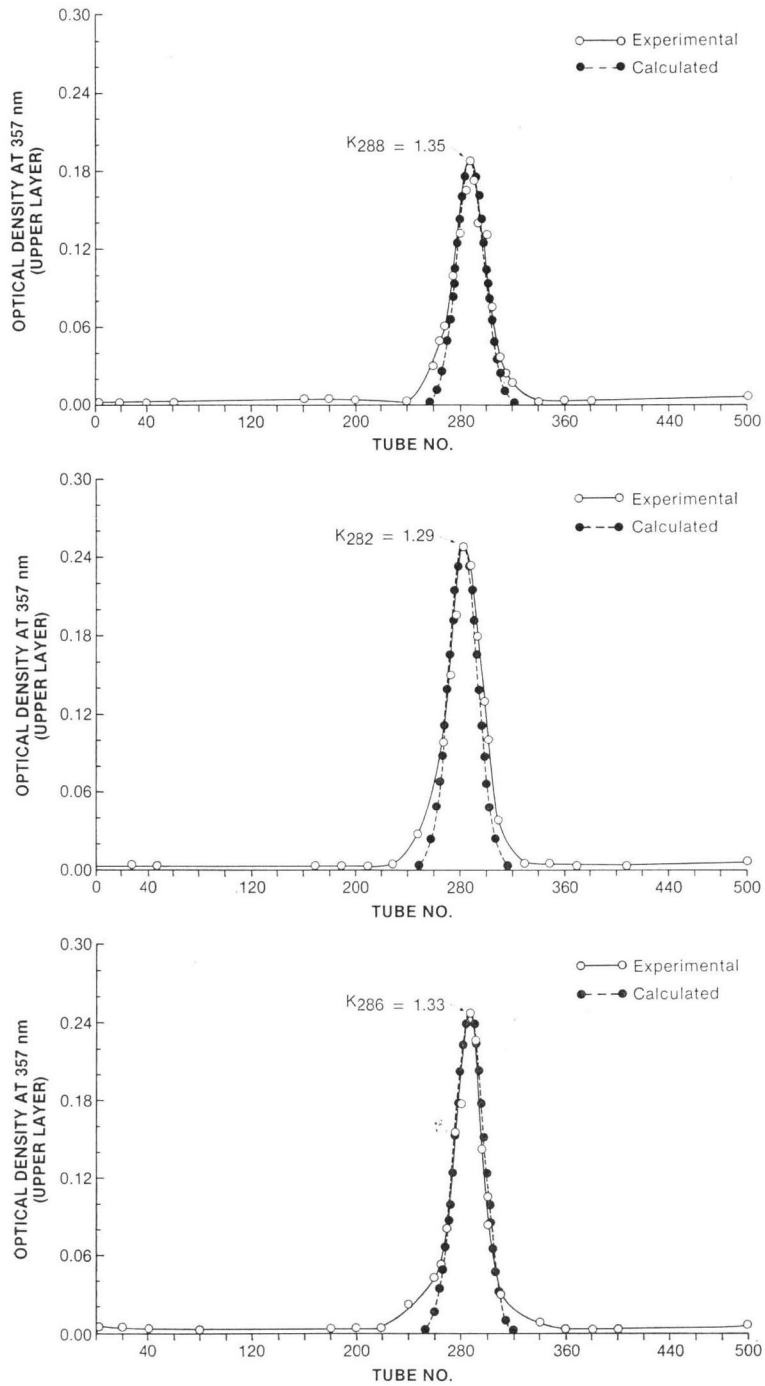
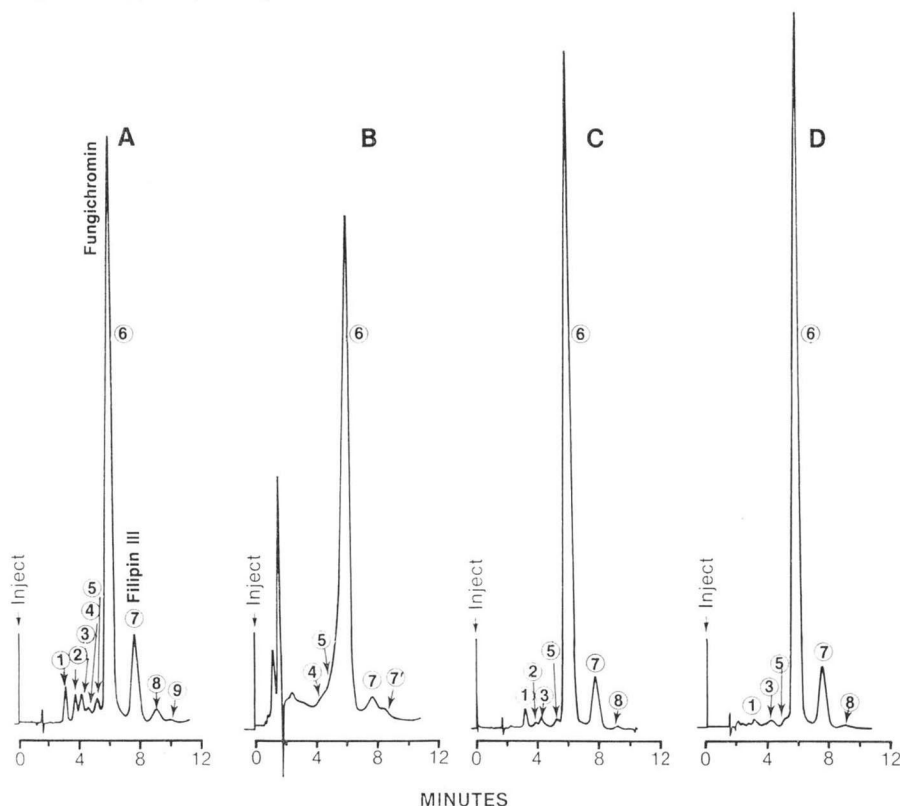


Fig. 2. High-performance liquid chromatograms: (A) fungichromin isolated at NCI-Frederick Cancer Research Facility, (B) authentic fungichromin, (C) lagosin, (D) cogomycin.

Conditions: C_{18} μ Bondapak column (3.9 mm \times 30 cm) UV (357 nm) detector, methanol - water (60:40) solvent system, 1.5 ml/minute flow rate.



^{13}C NMR and CD Studies

Because a detailed ^{13}C NMR study on cogomycin has already been reported in the literature⁽⁶⁾, we did a direct comparison only. Fig. 3 shows a comparison of the ^1H decoupled ^{13}C NMR spectra of purified fungichromin, lagosin, and cogomycin in pyridine- d_5 . There are no differences in the chemical shifts of the carbons among these antibiotics; we therefore believe that there are no stereochemical differences and that the physical differences observed previously were due to the impurities.

The CD spectra of purified fungichromin, lagosin and cogomycin are shown in Fig. 4.

Dose-response Patterns to Fungichromin, Lagosin and Cogomycin

Fig. 5 shows the dose-response patterns of RBC sensitivity to fungichromin, lagosin, and cogomycin. Leakage of K^+ paralleled hemolysis, and at no level of antibiotics were these effects separable.

The response of *S. cerevisiae* cells treated with fungichromin, lagosin, and cogomycin was similar in that K^+ leakage and loss of viability could not be separated (Fig. 6).

In contrast to the results reported for aureofungin B and other heptaene polyenes, no additional growth appeared after 42 hours of incubation. The effects of fungichromin, lagosin, and cogomycin were fungicidal and not fungistatic.

The results are summarized in Table 3.

Fig. 3. Comparison of ^1H decoupled ^{13}C NMR spectra (FX-60) of fungichromin (top), lagosin (center), and cogomycin (bottom) in pyridine- d_6 .

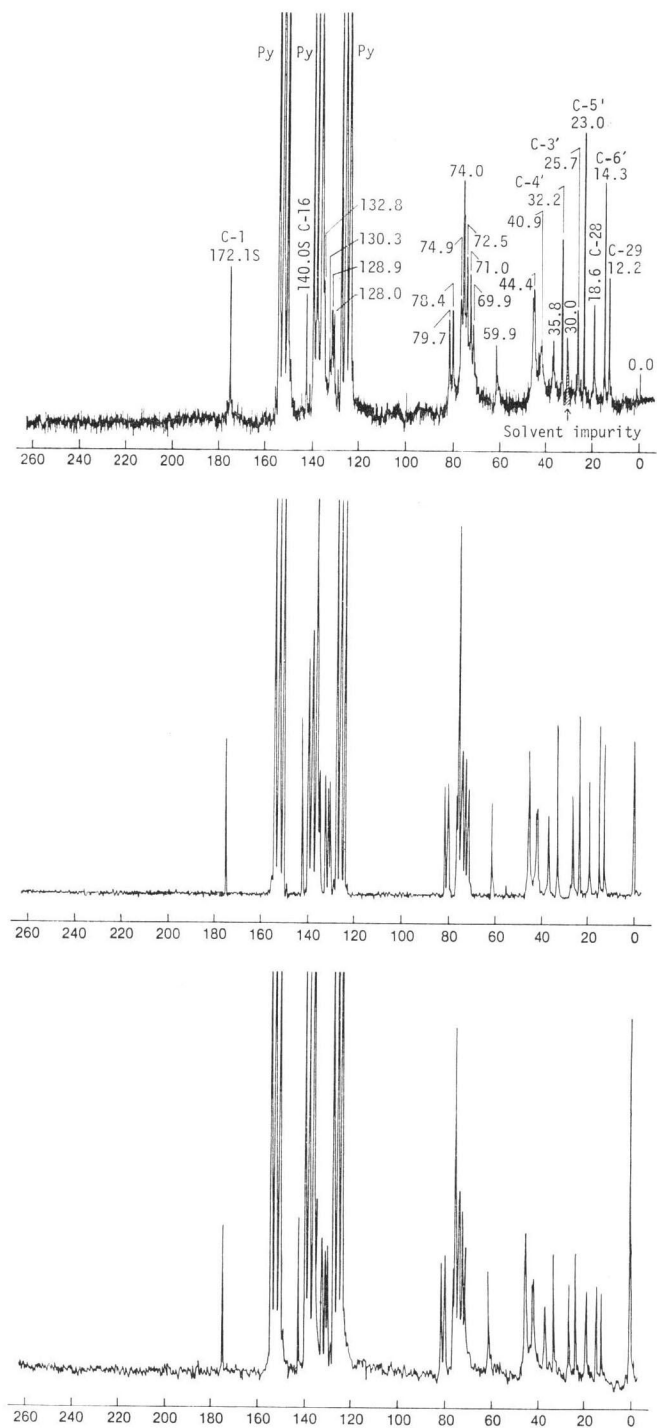


Fig. 4. Circular dichroism (CD) spectral comparison of fungichromin (FCRC-A21), lagosin, and cogomycin.

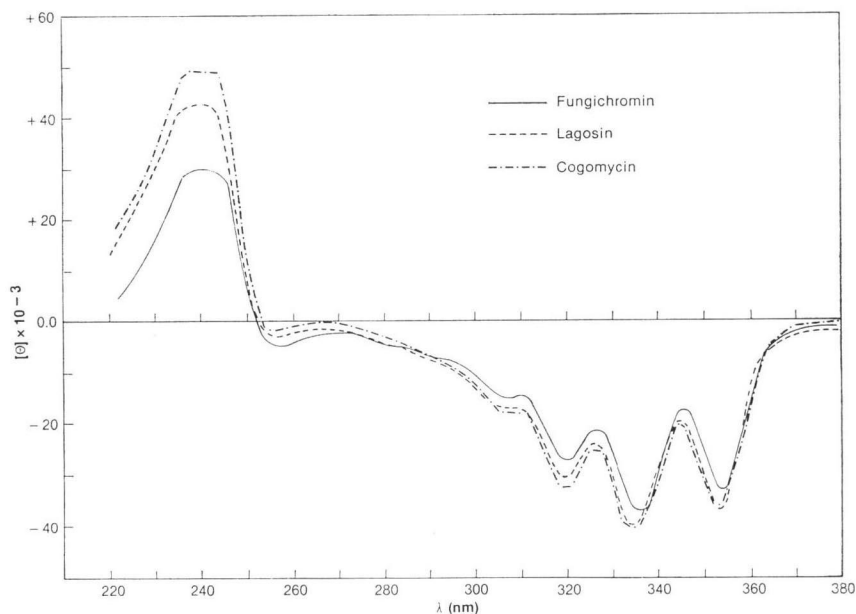
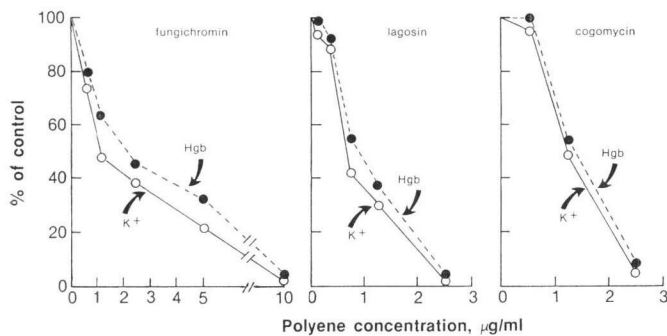


Fig. 5. Dose-response patterns of mouse erythrocytes to fungichromin, lagosin, and cogomycin.

Fig. 6. Dose-response of *Saccharomyces cerevisiae* to cogomycin.

The percent (%) of colony-forming units (cfu) at 42 hours and 72 hours was the same.

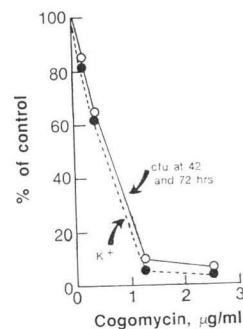


Table 3. Comparison of the concentrations of fungichromin, lagosin, and cogomycin required to cause 50% leakage of potassium with that required to cause 50% of either hemolysis (erythrocyte) or cell death (yeast).

Polyene	Erythrocyte			Yeast		
	Hgb ^a	K ⁺ ^a	Hgb/K ⁺ ^b	Death ^a	K ⁺ ^a	Death/K ⁺ ^c
Fungichromin	2.0	1.0	2.0	3.5	2.1	1.7
Lagosin	0.9	0.6	1.5	1.9	0.9	2.1
Cogomycin	1.3	1.4	1.1	0.6	0.5	1.2

^a=Micrograms per ml causing 50% effect. Hgb: Hemoglobin.

^b=Ratio of hemoglobin/K⁺ causing 50% effect.

^c=Ratio of cell death/K⁺ causing 50% effect.

Table 4. *In vivo* activity against P388 murine tumor cell lines of several pentaene preparations.*

Chainin		Filipin		Fungichromin		Cogomycin	
Dose (mg/kg)	T/C ⁺	Dose (mg/kg)	T/C ⁺	Dose (mg/kg)	T/C ⁺	Dose (mg/kg)	T/C ⁺
6	106	12	toxic	6	toxic	6	100
4	100	8	106	4	106	5	86
2	104	5	104	2	103	4	102
1	100	2.5	100	1	106		
0.5	100	1.0	108	0.5	103		

* Determined by Ms. PEG SHERIDAN, Battelle Laboratories, Columbus, Ohio.

⁺ The ratio of average survival of treated animals to that of controls in days T/C × 100.

In Vivo Activity against P388 Murine Tumor Cell Lines

In vivo P388 antitumor studies were also conducted on several other pentaene macrolide antibiotics. The results are summarized in Table 4.

Discussion

Even after repeated CCD purification of fungichromin, lagosin, and cogomycin in chloroform-methanol-borate buffer (2:2:1), it was not possible to remove all the minor components. As the purity increased, the properties of the antibiotics became very similar (compare Tables 1 and 2). The distribution coefficients (K) of fungichromin, lagosin, and cogomycin were identical; whereas, as expected, the distribution coefficient of filipin III, the major component of filipin complex, was much different (Table 2).

The results of HPLC of some of the polyene antibiotics have been reported in the literature¹²⁻¹⁵, and a fruitful comparison in identifying the various peaks has been made particularly in the group of heptaene macrolide antibiotics. In our studies, optimal separation of fungichromin, lagosin, and cogomycin from impurities was achieved using methanol-water (60:40) (Fig. 2) as the mobile phase. When the ratio of methanol to water was reduced, the peaks became very broad and the retention time increased; an increase in the ratio reduced the retention time, making the separation difficult. Fig. 2 clearly shows that the minor components are separable by this technique and that all of the antibiotics have one major component. The ¹³C NMR and CD studies were the most important of all the data with respect to determining the differences in the stereochemistry of fungichromin, lagosin, and cogomycin. The results of ¹³C NMR (Fig. 3) of the purified antibiotics in pyridine-*d*₅ were all superimposable, indicating that there were no stereochemical differences in the molecules. This result was further confirmed by the CD spectra (Fig. 4).

The samples of fungichromin, cogomycin and lagosin behaved similarly in regard to potency and pattern of biological action. Their activity was typical of a non-heptaene polyenes (Group I), as described earlier⁹ on the basis of the two characteristic indices. The first index compares the concentration of the antibiotic causing K⁺ leakage with that causing hemolysis of RBC (Hgb/K⁺) or yeast cell death (death/K⁺). The second index gives the number of colonies formed after 72 or 42 hours of plate incubation. No differences could be detected among the studied pentaene antibiotics. For the assayed antibiotics, both concentration ratios (Hgb/K⁺ and death/K⁺) were 2 or less and no growth inhibition (fungistatic effect) was observed.

The *in vivo* antitumor activity against P388 murine tumor cell lines (Table 4) indicates that these antibiotics exhibit toxicity at a relatively low dose level and have no therapeutic effect. These findings are in agreement with the mode of action of these antibiotics.

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