These findings suggest that the presence of the naphtholic OH group, as in I, is still necessary for biological activity within this group. Apparently, due to the possibility of chelation with the cations necessary for the metabolic functions of the parasite, the oxygen of this group and the nitrogen of the neighboring side chain may contribute in the cation complex formation.

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Synthesis and Biological Properties of Alkyl Esters of Polyene Antibiotics

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Abstract □ Several new alkyl esters of polyene antibiotics were prepared by an improved general procedure, and their toxicity and microbiological activity were tested. Some of these alkyl esters were more active than the nonesterified polyenes against *Candida albicans* but were less effective than the known methyl esters. Their toxicity was much less than that of the parent compounds.

Keyphrases □ Polyene antibiotics, alkyl esters—synthesis, antifungal activity □ Antifungal agents—synthesis and testing of alkyl esters of polyene antibiotics

The first attempt to reduce the toxicity of the macrolidic polyenes consisted of reacting the amino groups to give the corresponding *N*-acyl derivatives (1, 2), but these compounds were shown to be less active than the original antibiotics so chemotherapeutic employment was out of the question.

Since most natural polyenes have amphoteric characteristics, a new approach blocking the carboxyl group was developed and the first derivative, partricin methyl ester, was more active and less toxic than the parent antibiotic (3, 4). The methyl esters of other polyenes also gave good results (5, 6).

To continue this research, some homologous alkyl esters were prepared to determine whether other factors, such as the structure of the substituent group, could influence the biological activity of these polyene derivatives. The compounds were prepared by allowing the natural polyene to react with excess diazoalkane in the presence of ammonium hydroxide or other organic bases; surprisingly, the polyenes formed fewer by-products and thus biologically more active esters when in a basic medium.

The esterification of the carboxyl was confirmed by IR and NMR spectra, while TLC (Table I) on silica gel confirmed the quality of the products.

The polyene esters are pale-yellow to dark-yellow solids, which are practically insoluble in water, alkali, and the usual organic solvents and are very soluble in dimethyl sulfoxide, 2-methoxyethanol[1], and pyridine. The UV spectra show the typical absorbance patterns of the initial heptaenes and tetraenes (7).

All compounds were tested for their antifungal activity and acute toxicity in mice (LD_{50}); the results are reported in Table I in comparison with the parent substances and the known methyl esters. Since partricin is also active against protozoa, its esters were tested for their antitrichomonal activity in a preliminary manner.

EXPERIMENTAL

General Procedure of Synthesis—Concentrated ammonium hydroxide (0.5 ml) and then a 1.5% solution of diazoalkane in ether (10 ml) were added dropwise with stirring to a solution of polyene antibiotic (1 g) in dimethyl sulfoxide (20 ml). The mixture was kept at room temperature for 3 hr, and then ether (200 ml) was added to give a pasty solid. The precipitate was treated with acetone–ether, giving a high yield of the required ester.

TLC—TLC was carried out on silica gel[2] 60 F_{254}, using butanol–ethanol–acetone–32% ammonium hydroxide (2:5:1:3) as eluent and detecting the spots by exposure to UV light.

Antifungal Activity—The compounds were tested against *Candida albicans*, strain 200[3]. The test strain was cultured in Sabouraud medium[4] for 18 hr at 36° and diluted to 10% transmittance at 580 nm. Then about 0.15 ml of the diluted test strain was inoculated in 100 ml of Sabouraud medium so that the final test tubes contained 10^6 cells.

The substances were dissolved in dimethyl sulfoxide and serially diluted with sterile distilled water; 0.5 ml of each dilution was added to 4.5 ml of the inoculated broth, and all tubes were incubated for 24 hr at 36°. The results are reported as the minimum inhibitory concentration (MIC), *i.e.*, the lowest concentration of polyene antibiotic at which no visible growth was observed.

Acute Toxicity—The approximate LD_{50} was determined in groups of five female Swiss mice, 20–24 g. The compounds were

[1] Methyl cellosolve.
[2] Merck.
[3] Società Prodotti Antibiotici collection.
[4] Difco.

Table I—Alkyl Esters of Polyene Antibiotics

Compounds	R_f	MIC, μg/ml[a] (*C. albicans*)	Approximate LD_{50} (Mice) mg/kg ip[b]	mg/kg iv[c]
Partricin	0.51	0.125[d]	<5[e]	—
Partricin methyl ester	0.84	0.015 (0.062)[f]	200[g]	—
Partricin ethyl ester	0.88	0.015 (0.062)	100	—
Partricin propyl ester	0.90	0.031	200	—
Partricin butyl ester	0.92	0.062	>200	—
Amphotericin B	0.41	0.125	—	5
Amphotericin B methyl ester	0.79	0.062 (0.125)	—	75
Amphotericin B ethyl ester	0.82	0.125–0.250	—	>50
Amphotericin B propyl ester	0.84	0.250	—	>50
Amphotericin B butyl ester	0.87	0.250–0.500	—	>50
Candicidin	0.55	0.062	5	—
Candicidin methyl ester	0.86	0.062	75	—
Candicidin ethyl ester	0.89	0.125 (0.500)	200	—
Candicidin propyl ester	0.92	0.125	50	—
Candicidin butyl ester	0.92	0.250	100	—
Nystatin	0.42	1	150	—
Nystatin methyl ester	0.77	1	>200	—
Nystatin ethyl ester	0.79	2–4	>200	—
Nystatin propyl ester	0.80	4 (4)	>200	—
Nystatin butyl ester	0.81	8	>200	

[a] Values in parentheses refer to samples obtained in neutral medium. [b] Suspended in 0.5% carboxymethylcellulose. [c] Solubilized with sodium deoxycholate (1:1). [d] Lit. (8) MIC 0.2 μg/ml. [e] Lit. (8) LD_{50} 0.5 mg/kg. [f] Lit. (4) MIC 0.05 μg/ml. [g] Lit. (4) LD_{50} 200 mg/kg.

administered intraperitoneally (0.5% carboxymethylcellulose suspension, 20 ml/kg), except for amphotericin and its esters which had low toxicity in suspension (LD_{50} > 1000 mg/kg) and were tested intravenously after solubilization with sodium deoxycholate (1:1). Deaths were recorded after 7 days.

RESULTS AND DISCUSSION

The results indicate that all of the esters are antifungal and that some may be more active than the parent compounds. Nevertheless, the lengthening of the aliphatic chain in the ester group (methyl to butyl) produced a gradual decrease in activity.

On the whole, the highest increases in activity were obtained by esterifying the heptaenes, while the most active derivative (methyl ester) of the tetraene nystatin was only as potent as the natural product.

The best results in the heptaenes were provided by amphotericin (the activity of its methyl ester was doubled) and particularly by partricin. The methyl and ethyl esters of partricin were the most active, their MIC value against *C. albicans* being around 0.015 μg/ml and thus eightfold more active than the nonesterified product (MIC 0.125 μg/ml). In spite of the usual decrease in activity, the butyl ester also maintained a practically doubled activity.

The acute toxicity in mice by the intraperitoneal or intravenous route appeared to be considerably reduced with all alkyl esters, and there were no marked differences within each series of esters up to the maximum doses tested.

Any antitrichomonal activity possessed by the natural antibiotic (partricin MIC 0.25 μg/ml) seemed to be maintained (although slightly reduced) after esterification. No sensitive variation was seen in the series of the homologous esters that showed MIC values around 0.5–2 μg/ml.

To conclude, the blocking of the carboxyl of the amphoteric polyene antibiotics by alkyl esterification appears to diminish the toxicity while at the same time maintaining the microbiological activity. However, lengthening the alkyl chain provides less active esters.

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