HYDRAZIDES—A NOVEL TYPE OF DERIVATIVES OF POLYENE MACROLIDE ANTIFUNGAL ANTIBIOTICS

Sir:

Polyene macrolide antibiotics are important antimycotic agents. However, their use in therapy in the treatment of systemic mycoses is still limited due to the poor selective toxicity and low water solubility. Many efforts had been made to remove these undesirable features by modifications, but the problem of finding an effective drug has not been solved up to the present[1~10].

In our antifungal drug development program aimed at the improvement of the therapeutic properties of polyene macrolides we have established the structure-activity relationship among the derivatives of amphotericin B, the polyene antibiotic most commonly used in clinical practice[11]. It has been shown in these studies that the improvement of selective toxicity is related to the absence of a free carboxyl group in the modified antibiotic molecule. We have also demonstrated that water solubility of amphotericin B derivatives occurs only with net charged molecules.

In the present paper we report the novel group of polyene macrolide derivatives fulfiling the above requirements for improved selective toxicity and water solubility. These are polyene macrolide hydrazides formed in the reaction of carboxyl group activated antibiotics and 1-amino-4-methylpiperazine. The compounds obtained retain most of the antifungal activity of parent antibiotics and form with organic and inorganic acids salts with excellent water solubility at neutral pH. The compounds are also less hemolytic.

The polyene macrolide hydrazides were obtained in the reaction of 1-amino-4-methylpiperazine with the carboxyl group of amphoteric polyene antibiotics activated by the treatment with diphenyl phosphorazidate (DPPA)[12] and triethylamine. The reaction was carried out in DMF as a solvent using an excess of the reagents. The course of the reaction was followed by TLC on silica gel with the solvent system: 1-butanol - ethanol - acetone - ammonia aq (2 : 5 : 1 : 3). The crude product was isolated from the reaction mixture by precipitation with ethyl ether and partially purified using a 1-butanol - water extraction procedure. Final purification was achieved by ion-exchange chromatography on CM-52 cellulose or by counter-current distribution with a chloroform - methanol - 0.5% NaCl aq (2 : 2 : 1) solvent system.

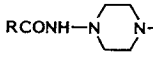
Hydrazides of polyene macrolides representing major structural groups, *i.e.*, amphotericin B, candidin, aureofacin and nystatin, were synthesized and characterized.

In a typical synthesis, to the solution of 1.38 g of amphotericin B in 30 ml of DMF cooled in an ice bath 1.98 ml of 1-amino-4-methylpiperazine, 2.1 ml of triethylamine and 3.48 ml of DPPA were added with stirring. The reaction mixture was left overnight at room temperature. The crude product was precipitated with an excess of ethyl ether, centrifuged and dissolved in H_2O - satd butanol. The organic layer was washed several times with water and concentrated in vacuum to a small volume. The derivative was precipitated with ethyl ether, centrifuged and dried under diminished pressure. For further purification the product was dissolved in H_2O - MeOH mixture (1 : 2) and charged on a column packed with CM-52 cellulose, washed with the solvent and eluted with a 5%-NaCl in MeOH - H_2O (2 : 1) solution. After evaporation of MeOH and dilution with H_2O followed by extraction with butanol in the presence of triethylamine, the butanol layer was washed with H_2O to remove NaCl (test with $AgNO_3$) and concentrated to a small volume. Precipitation with ethyl ether, washing and drying in vacuum yielded 0.9 g (60%) of the pure amphotericin B derivative.

The compound exhibited electronic absorption maxima of the same wavelength as amphotericin B at $\lambda = 363$, 382 ($E^{1\%}_{1cm}$ 1,320) and 406 nm in MeOH. The oscillation bands at $\nu = 1630\,cm^{-1}$ and lack of $1590\,cm^{-1}$ revealed in the IR spectrum documented the presence of an amide bond. In the positive ion FAB-MS of the derivative a *quasi*-molecular ion $(M+Na)^+$ at m/z 1,043 was observed. The most prominent ion at m/z $840(M+H-Su-H_2O)^+$ where Su is mycosamine sugar moiety/confirmed that the aglycone was modified upon antibiotic derivatization.

The biological properties of hydrazide derivatives are presented in Table 1. The concentrations of the substances tested inhibiting 50% of the growth of *Saccharomyces cerevisiae* ATCC 9763 and of a clinical isolate of *Candida albicans* (IC_{50}) and the concentrations producing 50% release of hemoglobin from human erythrocytes (EH_{50}) were taken as measures of the antifungal and hemolytic activities, respectively.

Attention should be paid to the derivative of

Table 1. Antifungal and hemolytic activities of  (R = polyene macrolide antibiotic moiety).

R	IC_{50} (μg/ml)		EH_{50} (μg/ml)
	S.c.	*C.a.*	
Amphotericin B	0.04[a]	0.03[a]	5[a]
	0.06	0.04	25
Candidin	0.05[a]	0.05[a]	20[a]
	0.20	0.20	100
Nystatin	0.12[a]	0.15[a]	50[a]
	0.35	0.25	>100
Aureofacin	0.003[a]	0.003[a]	0.5[a]
	0.005	0.0025	Agglutination

IC_{50}: The concentration of compound tested causing 50% inhibition of the growth of *S. cerevisiae* or *C. albicans* in Sabouraud liquid medium determined photometrically ($\lambda = 660$ nm) after 24 hours incubation at 28°C.

EH_{50}: The concentration of compound tested causing 50% release of hemoglobin from human erythrocytes in *iso*-osmotic (0.15 M) sodium chloride after 30 minutes incubation at 37°C, in standard conditions, determined photometrically at $\lambda = 550$ nm.

S.c.: *Saccharomyces cerevisiae* ATCC 9763; *C.a.*, *Candida albicans* (clinical isolate).

[a] Data for parent antibiotics.

amphotericin B which among polyenes still remains the only effective drug in the treatment of deep-seated mycoses and the main subject of extensive chemical modifications aimed at improvement of the selective toxicity index. The amphotericin B hydrazide exhibited most advantageous properties as compared to hydrazides of other polyenes. This compound was selected for pharmacological studies, which are under progress. For these studies highly water soluble diaspartate salt is used.

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JOLANTA GRZYBOWSKA
EDWARD BOROWSKI

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, 80-952 Gdańsk, Poland

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