N-DIMETHYLAMINOACYL DERIVATIVES OF POLYENE MACROLIDE ANTIBIOTICS

Sir:

The polyene macrolide antibiotics are clinically important antifungal agents. Because of the high animal toxicity and poor water solubility their application in chemotherapy is significantly limited. To improve biological and physicochemical properties the broad program of chemical modification of these antibiotics has been undertaken[1~9].

Here we present a new group of aminoacyl-type derivatives obtained in the *N*-acylation reaction of polyene macrolide antibiotics or their esters and amides with *N*,*N*-dimethylated amino acids.

When the native antibiotic was used as a substrate, the reaction was performed at room temperature in DMF with an excess of previously activated *N*,*N*-dimethylamino acid in form of *N*-hydroxysuccinimide ester in the presence of triethylamine (synthesis I).

In the case of antibiotic esters or amides the *N*-acylation with small excess of *N*,*N*-dimethylamino acid was carried out directly by means of azide method using diphenyl phosphorazidate (DPPA)[10] in the presence of triethylamine in DMF as a solvent at 0°C (synthesis II).

The course of the reactions was followed by thin-layer chromatography on silica gel with the solvent systems: $CHCl_3$ - MeOH - H_2O (10: 6: 1), butanol - EtOH - acetone - ammonia aq (2: 5: 1: 3) or $CHCl_3$ - MeOH - pyridine - 10% acetic acid aq (5: 3: 0.5: 0.5) depending on the antibiotic and its carboxylic substituent. The crude products were isolated from reaction mixtures by precipitation with ethyl ether, dissolved in butanol and washed with H_2O. The organic layer was concentrated *in vacuo* and ethyl ether was added to precipitate the desired derivatives. The compounds obtained were purified by counter-current distribution with the solvent system $CHCl_3$ - MeOH - 0.5% NaCl aq solution (2: 2: 1) or by gel filtration using Sephadex LH-20 in MeOH or by ion-exchange chromatography on CM-52 cellulose.

Derivatives of polyene macrolides representing major structural groups (amphotericin B, nystatin, aureofacin, perimycin) with *N*,*N*-dimethylglycine and *N*,*N*-dimethyl-β-alanine were synthesized and characterized. Also the same type of derivatives was obtained with polyenes methyl esters and 3-(*N'*,*N'*-dimethylamino)propyl amides.

In an example synthesis I, to 0.280 g of *N*,*N*-dimethylglycine hydrochloride in 6 ml of DMF 0.232 g of *N*-hydroxysuccinimide and 0.412 g of dicyclohexylcarbodiimide were added and left for 12 hours at room temperature. Dicyclohexylurea formed was filtered off, washed with the small volume of DMF and the filtrate was added to the solution of 0.923 g of amphotericin B and 0.42 ml of triethylamine in 15 ml of DMF. The reaction mixture was stirred for 12 hours at room temperature. The product was precipitated with an excess of ethyl ether, centrifuged and dissolved in H_2O - saturated butanol. The butanol layer was washed twice with H_2O and concentrated under reduced pressure. Precipitation with ethyl ether followed by washing with ether and drying *in vacuo* yielded 0.84 g (84%) of *N*-(*N'*,*N'*-dimethylglycyl)amphotericin B ($E^{1\%}_{1cm}$=1,420 at 382 nm in MeOH).

In a typical synthesis II, 0.937 g of amphotericin B methyl ester was dissolved in 15 ml of DMF and cooled to 0°C. Then 0.21 g of *N*,*N*-dimethylglycine hydrochloride, 0.42 ml of triethylamine and 0.35 ml of DPPA were added with stirring. After 5 hours product was precipitated with ethyl ether and isolated as described above. For purification the crude 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 5% NaCl in MeOH - H_2O (2: 1) solution. After evaporation of MeOH under reduced pressure and dilution with H_2O followed by extraction with butanol in the presence of triethylamine, the organic layer was washed with H_2O and concentrated to a small volume. Then the ethyl ether was added to precipitate the desired derivative. Thus, 0.715 g (70%) of *N*-(*N'*,*N'*-dimethylglycyl)amphotericin B methyl ester ($E^{1\%}_{1cm}$=1,300 at 382 nm in MeOH) was obtained.

Both example compounds obtained in syntheses I and II exhibited electronic absorption maxima of the same wavelength as amphotericin B itself at λ=363, 382 and 406 nm (MeOH). The oscillation bands at ν=1640 cm^{-1} revealed in the IR spectra documented the presence of an amide bond. Hydrolysis of derivatives under

Table 1. The antifungal and hemolytic activities of the *N*-dimethylaminoacyl derivatives of polyene macrolide antibiotics and their esters or amides.

Antibiotic	Compound	IC_{50} (μg/ml)		EH_{50} (μg/ml)
		S. cerevisiae	*C. albicans*	
Amphotericin B	Amphotericin B	0.05	0.03	5
	N-(*N'*,*N'*-Dimethylglycyl)amphotericin B	0.08	0.08	10
	N-(*N'*,*N'*-Dimethylglycyl)amphotericin B methyl ester	0.12	0.10	30
	N-(*N'*,*N'*-Dimethylglycyl)amphotericin B 3-(*N''*,*N''*-dimethylamino)propyl amide	0.13	0.12	25
	N-(*N'*,*N'*-Dimethyl-β-alanyl)amphotericin B methyl ester	0.10	0.12	20
Nystatin	Nystatin	0.15	0.15	50
	N-(*N'*,*N'*-Dimethylglycyl)nystatin methyl ester	0.32	0.25	>150
Aureofacin	Aureofacin	0.0002	0.0003	0.5
	N-(*N'*,*N'*-Dimethylglycyl)aureofacin 3-(*N''*,*N''*-dimethylamino)propyl amide	0.0002	0.0003	10
	N-(*N'*,*N'*-Dimethyl-β-alanyl)aureofacin methyl ester	0.0003	0.0003	8
	N-(*N'*,*N'*-Dimethyl-β-alanyl)aureofacin 3-(*N''*,*N''*-dimethylamino)propyl amide	0.0005	0.0004	7
Perimycin	Perimycin	0.0003	0.0003	>100
	N-(*N'*,*N'*-Dimethyl-β-alanyl)perimycin	0.0003	0.0003	6

IC_{50}: The concentration of compound tested causing 50% inhibition of the growth of *Saccharomyces cerevisiae* or *Candida albicans* in Sabouraud liquid medium determined photometrically (λ=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 λ=550 nm.

mild acidic conditions afforded *N*-(*N'*,*N'*-dimethylglycyl)mycosamine identified on the basis of its ^{1}H NMR spectrum and chromatographic comparison with authentic sample. Moreover, in the case of compound with a blocked carboxyl group, *i.e.* *N*-(*N'*,*N'*-dimethylglycyl)amphotericin B methyl ester, field desorption mass spectrometry was employed to confirm its molecular weight (*m*/*z* 1,022, M^+).

All the other compounds obtained were examined in the similar manner.

The biological properties of derivatives obtained are presented in Table 1. IC_{50} and EH_{50} values, respectively, express the activities of compounds tested towards *Saccharomyces cerevisiae* and *Candida albicans* used as models of fungi, and towards human erythrocytes representing the mammalian cells. The antifungal activities of *N*-dimethylaminoacyl derivatives of amphotericin B and nystatin and of their esters and amides are a few-fold lower as compared with parent antibiotics. Better retention of activity was shown by derivatives of both aromatic heptaenes examined. Simultaneously most of derivatives studied exhibit lower hemolytic activity.

The derivatives with substituted carboxyl group of polyene macrolides form with organic and inorganic acids at physiological pH the H_2O-soluble salts with retention of antifungal activity.

The comparison of the biological properties of the newly-described derivatives with those of parent antibiotics leads to the conclusion that the basic amino group of amino sugar moiety in these antibiotics, although essential for antifungal activity as reported previously[1,2], could be blocked by acyl substituents containing dimethylated amino group without any substantial loss of antibiotic activity. Apparently the newly introduced basic nitrogen atom from aminoacyl moiety takes over the function of amino group

of the parent antibiotic in the interaction with membrane components. The shift of basic nitrogen atom which still allows the compounds to retain the activity can be quite considerable, as not only α-aminoacyl but also β-aminoacyl derivatives exhibit the activity not essentially changed as compared to that of parent antibiotics. This conclusion is true also for results of WRIGHT *et al.*[9], who synthesized the related active derivatives of polyene macrolide antibiotics but with free amino group in acyl residue. In our case, in contrary to these author's synthesis, there is no need to protect the amino group in amino acid before antibiotic acylation.

The retainment of biological activity of dimethylaminoacyl derivatives of polyene macrolides corroborates another general observation that although for the high biological activity the basic character of nitrogen atom is indispensable, the protons of amino group do not play any essential role and can be replaced by alkyl substituents. *N*,*N*,*N*-Trimethylammonium derivatives of polyenes exhibit unchanged activity[6]. The same is true for the presently described compounds with shifted amino group.

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