SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF AMIDES OF AMPHOTERICIN B

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A series of aliphatic amides of amphotericin B have been synthesized. The structure of the derivatives which were obtained has been established by mass spectrometry. Their biological properties: inhibition of growth of *Saccharomyces cerevisiae* and hemolytic activity have been determined. The quantitative relationships between the activity of amides of amphotericin B against *S. cerevisiae* and their lipophilicity can be expressed by a parabolic function, whereas that between hemolytic activity and lipophilicity—by polynominal expression of fourth degree.

Amphotericin B is commonly applied in the therapy of fungal infections. Of particular value is the usefullness of this antibiotic in the treatment of systemic mycoses. Nevertheless, its clinical use is limited due to the side effects accompanying the infusion of the drug.

A number of derivatives of amphotericin B have been synthesized in order to improve the pharmacological properties of the antibiotic[1~6]. None of these fulfilled all the requirements of an effective therapeutic agent; therefore, the search for new derivatives and their evaluation is still underway. Application of quantitative methods of structure-biological activity relationships should rationalize this search.

The present paper deals with the preparation and identification of aliphatic amides of amphotericin B, as well as analysis of the relationships between their structure and biological properties.

Experimental

Instrumental Analysis

The mass spectra were obtained on a Varian MAT 711 double focusing spectrometer by means of a direct introduction probe. The instrumental conditions were as follows: a) electron impact mode-electron energy 70 eV, emission current 0.8 mA, accelerating voltage 8 kV, ion source temperature 250°C, resolution (10% valley definition) 1,000; b) field desorption mode-wire heating current 18~21 mA, extraction voltage 4 kV.

The infrared and electronic spectra were recorded on UR-10 and ultraviolet-visible Carl Zeiss Jena instruments, respectively.

General Procedure of Synthesis of Amides of Amphotericin B

In general, 930 mg (1 mmole) of amphotericin B (E. R. Squibb and Sons, batch 91830-001, assay 97%) was suspended in 20 ml of *N*,*N*-dimethylacetamide, stirred at room temperature and treated with 10 mmoles of triethylamine, 10 mmoles of appropriate amine and 10 mmoles of diphenyl phosphorazidate. The course of the reaction was followed by means of thin-layer chromatography on DC-Alufolien Kieselgel 60 F_{254} (Merck) using solvent systems: ethyl acetate - acetic acid - water, 4: 1: 1 (v/v/v) or chloroform - methanol - water, 20: 10: 1 (v/v/v). After completion of the reaction the curde product was precipitated with 300 ml of ether, centrifuged, dissolved in 1-butanol and washed twice with water. After azeotropical evaporation of 1-butanol and water under reduced pressure, the prod-

uct was precipitated with ethyl ether, centrifuged, washed three times with ethyl ether and hexane and dried in vacuum. The derivatives were purified by means of counter-current distribution (chloroform - methanol - water, 2: 2: 1 (v/v/v), 150~200 transfers). The purity of the derivatives which were prepared was controlled by means of thin-layer chromatography and on the basis of their extinction coefficients in methanol at 382 nm.

The Silylation Procedure

In all, 5 mg of the compound to be analyzed was suspended in 1 ml of toluene - *N*-trimethylsilylimidazole, 10: 1 (v/v), and left overnight. The solvents were removed under reduced pressure, 4 ml of heptane was added to the residue and the resultant solution was washed twice with water, dried over magnesium sulfate and centrifuged. The supernatant was then concentrated to 0.2 ml and directly used for the mass spectroscopic measurements.

N-Acetylamphotericin B *n*-Hexyl Amide

Here, 200 mg of amphotericin B *n*-hexyl amide was dissolved in 5 ml of methanol - *N*,*N*-dimethylformamide, 1: 1 (v/v), cooled to 0°C, and with stirring 30 μl of triethylamine and 25 μl of acetic anhydride were added. The reaction was completed after 1 hour (TLC on silica gel, solvent system: ethyl acetate - acetic acid - water, 4: 1: 1 (v/v/v). Then, 1 ml of 1-butanol was added and the methanol was evaporated under reduced pressure. The product was precipitated with ethyl ether, washed with ethyl ether and hexane, centrifuged, and dried in vacuum. (Yield; 190 mg, $E^{1\%}_{1cm}$ 1300 at 382 nm in methanol, calculated purity 92%).

N-Acetyltetradecahydroamphotericin B *n*-Hexyl Amide

To a stirred suspension of 150 mg of *N*-acetylamphotericin B *n*-hexyl amide in 150 ml of methanol 10 mg of 10% $Pd/BaSO_4$ was added and the mixture was saturated with hydrogen for 16 hours. The catalyst was centrifuged and to the supernatant 5 ml of 1-butanol was added and methanol was evaporated under reduced pressure. The crude derivative was precipitated with hexane, centrifuged, washed with hexane, and dried in vacuum. The product was purified by means of chromatography on silica gel with the solvent system: chloroform - methanol - water, 500: 80: 6 (v/v/v). Yield; 90 mg.

N-Acetyl-13-methoxyiminotetradecahydroamphotericin B *n*-Hexyl Amide

To a solution of 50 mg of *N*-acetyltetradecahydroamphotericin B *n*-hexyl amide in 2 ml of pyridine 17 mg of methoxyamine hydrochloride was added and left overnight at room temperature. The mixture was poured into 6 ml of 1-butanol. The resultant organic layer was washed three times with water - acetic acid mixture 20: 1 (v/v) and concentrated under reduced pressure. The product was precipitated with ethyl ether - hexane, 1: 1 (v/v) centrifuged, washed with hexane, and dried in vacuum. Yield; 25 mg.

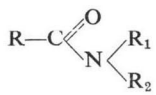
Determination of Biological Properties

The procedures for the determination of the biological properties of the antibiotic derivatives have been applied according to CYBULSKA *et al.*[7].

The antifungal activity of amides of amphotericin B was determined against *Saccharomyces cerevisiae* ATCC 9763. The nutrient medium: glucose 2%, Bacto-peptone (Difco) 1%, sodium chloride 0.5% was inoculated with yeast in the ammount 1 mg of dry weight per 1,000 ml. The substances to be tested were dissolved in dimethylsulfoxide at milimolar concentrations. The resultant solutions were prepared on the basis of the extinction coefficients, and further diluted with sterile water. In all, 0.5 ml of the inoculated medium and 0.5 ml of the antibiotic solution were incubated for 24 hours at 29°C, and thereafter the extent of growth of the yeast cells was determined spectrophotometrically at 660 nm. The concentrations of the antibiotic substances at which the growth of yeast was inhibited by 50% (IC_{50}) were determined from the growth curves and were assumed to be a measure of the antifungal activity (Table 1).

The hemolytic activity was determined as that concentration of the antibiotic derivative producing a 50% lysis of human erythrocytes (EH_{50}; Table 1). Erythrocytes isolated from fresh citrated human blood were washed twice with cold saline and once with isotonic choline chloride bufferd with 3 mM tris-HCl, pH 7.5. The cells were diluted 250 times with saline and equilibrated for 30 minutes at 20°C.

Table 1. *In vitro* biological properties of amphotericin B amides.



Compound	R_1	R_2	*Saccharomyces cerevisiae*			Hemolytic activity			Selective toxicity *in vitro* $ST=EH_{50}/IC_{50}$		
			IC_{50} (mcg/ml)	pIC_{50}		EH_{50} (mcg/ml)	pEH_{50}		ST	log ST	
				Obsd.	Calcd.[a]		Obsd.	Calcd.[b]		Obsd.	Calcd.[c]
1	H	$-CH(CH_3)_2$	0.072	1.14	1.28	5.12	−0.71	−0.71	70.6	1.85	2.02
2	H	$-CH_2CH_2CH_2CH_3$	0.068	1.17	1.17	6.02	−0.78	−0.76	89.0	1.95	1.93
3	H	$-CH_2CH(CH_3)_2$	0.063	1.20	1.20	5.36	−0.73	−0.75	85.0	1.93	1.95
4	H	$-CH_2CH_2CH_2CH_2CH_2CH_3$	0.104	0.98	0.88	5.75	−0.76	−0.78	54.8	1.74	1.65
5	H	$-cyclo-C_6H_{11}$	0.085	1.07	1.07	5.48	−0.74	−0.77	64.5	1.81	1.84
6	H	$-(CH_2)_9CH_3$	1.12	−0.05	−0.03	4.89	−0.69	−0.65	4.4	0.64	0.63
7	H	$-(CH_2)_7CH_3$	0.323	0.49	0.48	5.88	−0.77	−0.73	18.2	1.26	1.22
8	H	$-CH_2CH_2CH_2OH$	0.041	1.39	1.39	3.98	−0.60	−0.58	97.5	1.99	2.03
9	H	$-CH_2CONH_2$	0.063	1.20	1.18	3.62	−0.56	−0.56	57.4	1.76	1.73
10	H	$-CH_2CH_2CH_2COOCH_3$	0.071	1.15	1.17	7.22	−0.86	−0.86	102	2.01	2.03
11	H	$-CH_2CH_2CH_2N(CH_3)_2$	0.120	0.92	0.94	14.1	−1.15	−1.14	117	2.07	2.03
12	H	$-CH_2CH_2CH_2NH_2$	0.093	1.03	1.01	10.7	−1.03	−1.04	115	2.06	2.03
13	H	$-(CH_2)_{11}CH_3$	4.66	−0.67	−0.60	3.30	−0.52	−0.56	0.7	−0.15	−0.11
14	H	$-CH_2CH_2OH$	0.034	1.47	1.40	3.16	−0.50	−0.51	93.1	1.97	1.97
15	$-CH_2CH_2CH_2CH_2CH_2-$		0.144	0.84	0.83	12.8	−1.11	−1.13	89.0	1.95	1.92
16	$-CH_2CH_2OCH_2CH_2-$		0.093	1.03	1.04	10.7	−1.03	−1.01	115	2.06	2.05
17[d]	H	$-(CH_2)_{17}CH_3$	inactive	—	—	12.6	−1.10	−1.10	—	—	—

a Calculated by equation 1. b Calculated by equation 7. c Calculated by equation 8. d Compound omitted in the development of equations: 1 ~ 6 and 8.
R Unreacted part of amphotericin B.
$pIC_{50}=-\log IC_{50}$
$pEH_{50}=-\log EH_{50}$

Table 2. Physicochemical parameters of amphotericin B amides.

Compound	$\Sigma\pi$	ΣE_s	$\Sigma\sigma^*$	D_d	D_n	D_c	D_o
1	1.37	0.77	0.30	0	0	0	0
2	2.00	0.85	0.36	0	0	0	0
3	1.87	0.31	0.365	0	0	0	0
4	3.00	0.84	0.36	0	0	0	0
5	2.39	0.45	0.34	0	0	0	0
6	5.00	0.91	0.36	0	0	0	0
7	4.00	0.91	0.36	0	0	0	0
8	0.34	0.84	0.56	0	0	0	1
9	−1.21	0.86	1.20	0	0	1	0
10	1.23	0.84	0.58	0	0	1	0
11	1.18	0.84	0.52	0	1	0	0
12	0.31	0.85	0.52	0	1	0	0
13	6.00	0.91	0.36	0	0	0	0
14	−0.16	0.88	0.69	0	0	0	1
15	2.05	−0.43	−0.14	1	0	0	0
16	0.77	−0.43	−0.67	1	0	0	0
17	9.00	0.91	0.36	0	0	0	0

Here, 2 ml aliquots of this cell suspension were supplemented with various concentrations of substances to be tested and the incubation was continued with shaking for 30 minutes at 20°C. The lysis of erythrocytes was followed by measurement of the hemoglobin released into the solution after removal of the intact cells by centrifugation. The degree of hemolysis was calculated according to the formula:

$$\% \text{ of hemolysis} = 100 \times E_H/E_K ,$$

where E_H represents the optical density measured at 550 nm of the supernatant of the antibiotic-cell reaction mixture and E_K represents the optical density at the same wavelength of a supernatant of completely hemolysed erythrocytes. The concentrations, EH_{50}, of the antibiotic substances causing in a 50% lysis of the erythrocytes were determined from the hemolysis curves.

The ratio of EH_{50} and IC_{50} was taken as the measure of the selective *in vitro* toxicity of the amides of amphotericin B (Table 1).

Structure-Activity Relationships Methods

The HANSCH's method[8] had been applied in order to study the quantitative structure-activity relationships. Physicochemical parameters of the amides of amphotericin B tested are given in Table 2. The values of parameters: π, σ^* and E_s for substituents R_1 and R_2 had been taken directly from the literature or calculated on the basis of these data[8~14]. The summarized values of parameters of substituent R_1 and R_2: $\Sigma\pi$, $\Sigma\sigma^*$ and ΣE_s were taken as the measure of lipophilicity, electronic and steric properties of the amides of amphotericin B tested, respectively.

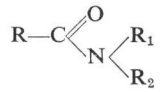
The presence of functional groups: amine, hydroxyl and carboxylic in the substituent R_2 as well as the presence of tertiary amide were described by parameters: D_n, D_o, D_c and D_d, respectively (Table 2).

The equations were obtained using the method of least squares and calculated on an ODRA 1204 computer.

Results and Discussion

The preliminary data on the synthesis and properties of amides of polyene macrolide antibiotics had been previously reported[15]. The amides of amphotericin B were obtained according to the general procedure based on the reaction of the antibiotic in the *N,N*-dimethylacetamide with the appropriate amine in the presence of diphenyl phosphorazidate and triethylamine.

Table 3. The structure and spectroscopic data of amides of amphotericin B.



Compound	R_1	R_2	$E^{1\%}_{1cm}$	Calculated purity (%)	$\nu_{c=o}$ cm^{-1}
1	H	$-CH(CH_3)_2$	1300	85	1640
2	H	$-CH_2CH_2CH_2CH_3$	1290	85	1640
3	H	$-CH_2CH(CH_3)_2$	1420	94	1640
4	H	$-CH_2CH_2CH_2CH_2CH_2CH_3$	1320	90	1640
5	H	-cyclo-C_6H_{11}	1350	92	1640
6	H	$-(CH_2)_9CH_3$	1160	83	1640
7	H	$-(CH_2)_7CH_3$	1140	80	1640
8	H	$-CH_2CH_2CH_2OH$	1400	93	1640
9	H	$-CH_2CONH_2$	900	60	1675
10	H	$-CH_2CH_2CH_2COOCH_3$	1100	76	1645
11	H	$-CH_2CH_2CH_2N(CH_3)_2$	970	66	1640
12	H	$-CH_2CH_2CH_2NH_2$	1000	66	1640
13	H	$-(CH_2)_{11}CH_3$	1040	78	1640
14	H	$-CH_2CH_2OH$	1070	70	1645
15	$-CH_2CH_2CH_2CH_2CH_2-$		1130	78	1600
16	$-CH_2CH_2OCH_2CH_2-$		1180	79	1600
17	H	$-(CH_2)_{17}CH_3$	780	62	1640

Where:

R represent the remaining part of molecule of amphotricin B.

$E^{1\%}_{1cm}$ extinction coefficients in methanol at 382 nm.

$\nu_{c=o}$ wavelength of bands characteristic for carbonyl bonds in oscillation spectra.

Amphotericin B was substituted with various aliphatic amines in order to establish the relations between the physico-chemical properties of the derivative and their biological activity. The chain length of the applied amines differed from one carbon unit up to eighteen. The additional functional groups, which were introduced with the amine substituent, included the following: hydroxyl, substituted amino, carboxyl and phosphonic.

The derivatives obtained and their spectroscopic data are listed in the Table 3.

The electron absorption spectra of amphotericin B amides were identical to that of the parent antibiotic. The oscillation spectra exhibited bands of significant intensities at $1640 \sim 1675\ cm^{-1}$ (for secondary amides) and at $1600\ cm^{-1}$ (for tertiary amides), whereas band of carboxylate at $1590\ cm^{-1}$ characteristic for the parent compound was not present in the spectra of the analyzed compounds.

The field desorption mass spectra of a number of representative amides of amphotericin B are listed in the Table 4. They displayed prominent ions in the molecular region. In the spectra detected were the molecular ions (M^+) and ions formed as a result of loss of molecules of water ($M^+ - n \times H_2O$). This indicates the utility of the derivatives obtained for determination of molecular weights of the parent antibiotics.

A detailed mass spectroscopic identification procedure had been performed for amphotericin B n-hexyl amide (**I**). The derivative **I** was *N*-acetylated in the reaction with acetic anhydride in methanol (**II**) and the product further transformed by reduction with hydrogen in the presence of palladium cata-

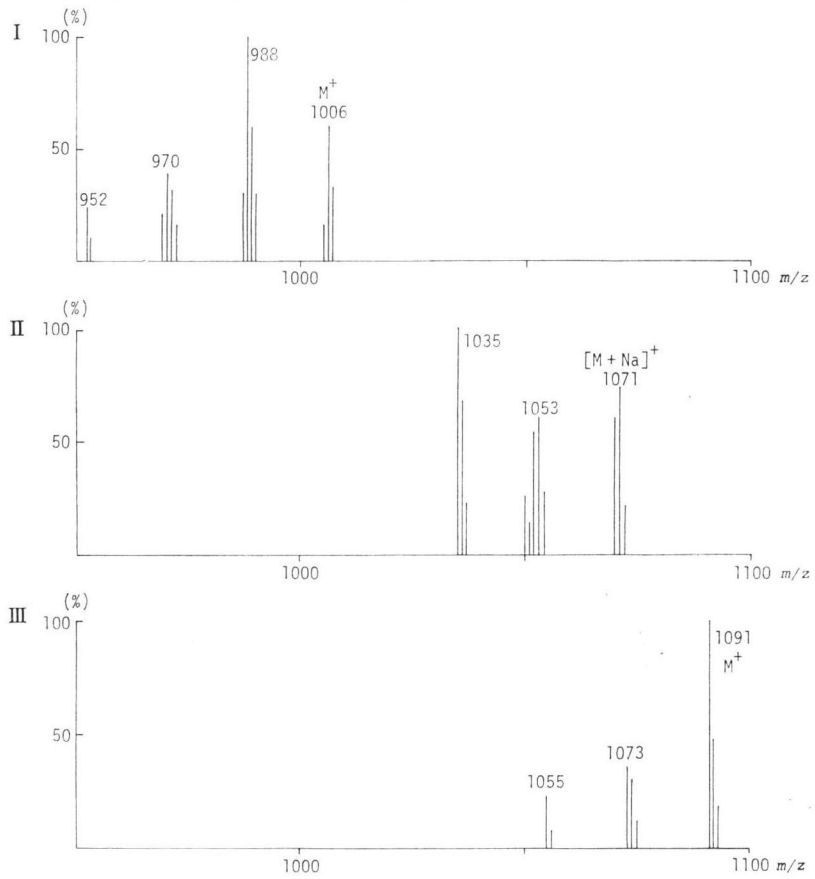
Table 4. Field desorption mass spectra of amides of amphotericin B.

Number of compound from Table 1	M. (calculated)	Ions (m/z) and relative intensities (into brackets)*
1	964	965(33%), 964(M^+; 53%), 963(28%), 947(40%), 946($M^+ - H_2O$; 100%), 945(50%), 928($M^+ - 2\times H_2O$; 32%)
3	978	979(71%), 978(M^+; 100%), 977(50%), 961(36%), 960($M^+ - H_2O$; 43%)
4	1006	1007(32%), 1006(M^+; 59%), 990(30%), 989(60%), 988($M^+ - H_2O$; 100%), 987(31%), 971(32%), 970($M^+ - 2\times H_2O$; 39%)
5	1004	1006(50%), 1005(57%), 1004(M^+; 100%), 1003(43%), 988(29%), 987(32%), 986($M^+ - H_2O$; 39%), 987(25%)
6	1062	1064(45%), 1063(82%), 1062(M^+; 100%), 1045(27%), 1044($M^+ - H_2O$; 45%), 1026($M^+ - 2\times H_2O$; 27%)
11	1007	1009(31%), 1008(82%), 1007(M^+; 100%), 990(25%), 989($M^+ - H_2O$; 28%)

* Ions of relative intensities above 25% were taken into consideration.

Fig. 1. Field desorption mass spectra.

I. Amphotericin B *n*-hexyl amide (M=1,006).
II. *N*-Acetylamphotericin B *n*-hexyl amide (M=1,048).
III. *N*-Acetyl-13-methoxyiminotetradecahydroamphotericin B *n*-hexyl amide (M=1,091).



lyst, followed by treatment with *O*-methylhydroxylaminehydrochloride in pyridine to produce the 13-methoxyiminotetradecahydro derivative (**III**). The field desorption spectra of the derivatives **I**~**III** are shown on Fig. 1. The electron impact mass spectra were taken for the persilylated derivatives of **II**

Fig. 2. Electron impact mass spectrum of deca-*O*-trimethylsilyl-*N*-acetylamphotericin B *n*-hexyl amide (M=1,768).

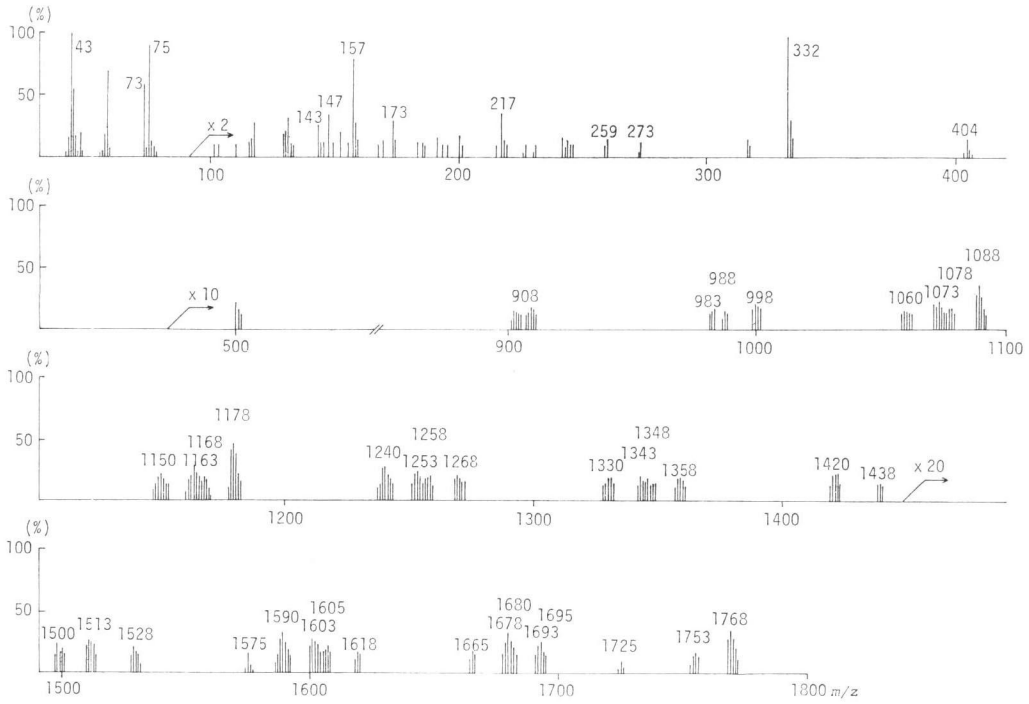
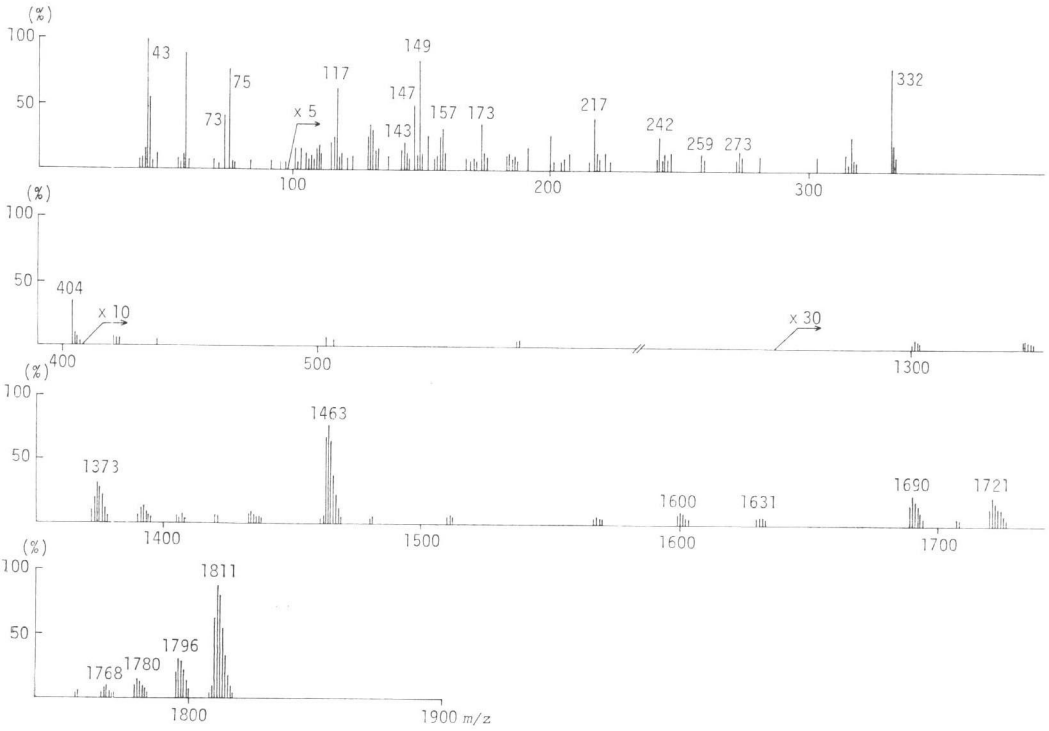


Fig. 3. Electron impact mass spectrum of deca-*O*-trimethylsilyl-*N*-acetyl-13-methoxyiminotetradecahydroamphotericin B *n*-hexyl amide (M=1,811).



and **III** (Figs. 2 and 3, respectively), which were obtained in the reaction with *N*-trimethylsilylimidazole in toluene. As the main diagnostic peaks we considered the following: molecular ions (at *m/z* 1,768 and 1,811, respectively), ions formed as a result of the loss of molecules of trimethylsilanol and of the amino-sugar moiety at *m/z* 1,420 and 1,463, respectively. The series of ions: *m/z* 143, 157, 259 and 273 confirmed the presence of a lactone bond[16]. The most prominent ions in the spectra of the deca-*O*-trimethylsilyl derivatives of *N*-acetylamphotericin B *n*-hexyl amide (**IV**) and of *N*-acetyl-13-methoxyiminotetradecahydroamphotericin B *n*-hexyl amide (**V**) were derived from the substituted mycosamine moiety: *m/z* 332, 242, 217, 147, and 143.

The amides of amphotericin B form with inorganic and organic acids salts which are soluble in water. The polyene macrolide amide formed in the reaction with 3-(*N*,*N*-dimethylamine)-*n*-propylamine, in particular, exhibit excellent solubility in water.

Yeasts and erythrocytes containing in the plasma membranes ergosterol and cholesterol, respectively, were used as the model cells.

The antifungal activity of amphotericin B amides ranged from those, which are equal to the parent antibiotic (for example: amphotericin B 2-hydroxyethyl amide) to those which are practically inactive (amphotericin B *n*-octadecyl amide). All the derivatives synthesized exhibited lower hemolytic activities as compared with the parent antibiotic.

The equations optimally characterizing the relationship between the physico-chemical properties of amphotericin B amides and the activities against *S. cerevisiae* are shown in Table 5. The correlations obtained interpret about 98% of the variance of biological data. Statistically most significant, according to the F test, is the equation 1. It reveals a high correlation between the antifungal activity of compounds tested and the lipophilicity of the substituents. This dependence can be illustrated as a parabolic function. The ideal lipophilic value (π_0) predicted from that equation is equal to 0.00 corresponding to, *e.g.*, amphotericin B amide. The calculation of the maximum pIC_{50} value of the aliphatic amide derivatives based on the π_0 value indicate, that all the derivatives should be equal or less active than the parent antibiotic. Additional functional groups, like amino, carboxylic and tertiary amide result in an additional decrease of the antifungal activity.

Equations representing the relationship between structure of amphotericin B amides and their hemolytic activity are shown in Table 6. The statistically most significant equation 4 explains about 99% of the variance of the biological activity. The hemolytic activity of the derivatives depends slightly on the lipophilicity whereas strongly on the presence of an additional amino, carboxylic or disubstituted amide

Table 5. Equations generated for activities against *S. cerevisiae* of amphotericin B amides.

Eq No.	Equation	n	s	r	F
1	$pIC_{50}=1.398(\pm0.056)-0.057(\pm0.004)\ (\Sigma\pi)^2-0.326(\pm0.106)\ D_d$ $-0.381(\pm0.108)\ D_n-0.138(\pm0.107)\ D_c$	16	0.060	0.996	311.30
2	$pIC_{50}=1.200(\pm0.089)-0.057(\pm0.005)\ (\Sigma\pi)^2+0.208(\pm0.120)\ \Sigma E_s$ $-0.359(\pm0.157)\ D_n$	16	0.089	0.989	185.26
3	$pIC_{50}=1.247(\pm0.067)-0.056(\pm0.004)\ (\Sigma\pi)^2+0.310(\pm0.130)\ \Sigma\sigma^*$ $-0.392(\pm0.133)\ D_n-0.265(\pm0.152)\ D_c$	16	0.073	0.994	211.24

Where: $pIC_{50}=-\log IC_{50}$
n number of compounds included into calculation.
s standard deviation.
r correlation coefficient.
F value of F test for generated equation.

Table 6. Equations generated for hemolytic activities of amphotericin B amides.

Eq No.	Equation	n	s	r	F
4	$pEH_{50}=-0.553(\pm0.032)-0.145(\pm0.022)\Sigma\pi+0.024(\pm0.004)(\Sigma\pi)^2-0.372(\pm0.045)D_d-0.448(\pm0.046)D_n-0.192(\pm0.052)D_e$	16	0.025	0.995	194.60
5	$pEH_{50}=-0.648(\pm0.052)-0.150(\pm0.023)\Sigma\pi+0.026(\pm0.004)(\Sigma\pi)^2+0.457(\pm0.065)\Sigma E_s-0.409(\pm0.121)(\Sigma E_s)^2-0.443(\pm0.049)D_n-0.191(\pm0.053)D_e$	16	0.025	0.995	162.81
6	$pEH_{50}=-0.593(\pm0.097)+0.016(\pm0.004)(\Sigma\pi)^2-0.239(\pm0.153)\Sigma\sigma^*-0.214(\pm0.069)\Sigma\pi\cdot\Sigma\sigma^*-0.699(\pm0.194)D_d-0.302(\pm0.064)D_n+0.199(\pm0.065)D_e$	16	0.034	0.992	87.38
7	$pEH_{50}=-0.5361(\pm0.0380)-0.1403(\pm0.0232)\Sigma\pi+0.0094(\pm0.0015)(\Sigma\pi)^3-0.0009(\pm0.0001)(\Sigma\pi)^4-0.3704(\pm0.0520)D_d-0.4564(\pm0.0538)D_n-0.1708(\pm0.0693)D_e$	17	0.029	0.994	139.85

Where: $pEH_{50}=-\log IC_{50}$
s standard deviation.
F value of F test for generated equation.
n number of compounds included into calculation.
r correlation coefficient.

groups. Equation 7 calculated for the parameters of amphotericin B *n*-octadecyl amide describes the hemolytic activity as a polynominal of fourth degree function of parameter π as indicates the presence of a minimum.

The relationship between the structure of amphotericin B amides and their selective *in vitro* toxicity can be characterized by equation 8:

(8) $\lg ST=1.995(\pm0.050)+0.125(\pm0.043)\Sigma\pi+-0.079(\pm0.008)(\Sigma\pi)^2$

$n=16$, $r=0.996$, $s=0.060$, $F=778.64$

The selective toxicity of the derivatives could be described as a parabolic function of the parameter π. That means the lipophilicity of the substituents R_1 and R_2 determines the ST values, whereas the parameters σ^* and E_s do not have significant influence on its level. The predicted optimal π_0 value is equal to $0.79(\pm0.35)$ which corresponds to amphotericin B morpholine or 2-(*N*,*N*-dimethylamino)ethyl amides. The calculated maximum ST value ($\lg ST=2.05\pm0.13$) is two and half times higher than that for the parent antibiotic ($\lg ST=1.67\pm0.18$). The additional functional groups introduced to amide substituents do not influence the selective *in vitro* toxicity.

The results obtained point to the strategy for search of new derivatives of polyene macrolides exhibiting improved biological properties as compared with the parent antibiotics.

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