AMPHOTERICIN B *O*-METHYL OXIME

SYNTHESIS AND BIOLOGICAL PROPERTIES

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Synthesis and biological properties of amphotericin B *O*-methyl oxime are described. The presence of an intact hemiketal ring in the antibiotic molecule appeared to be essential for its biological activity.

Amphotericin B (Fig. 1) is the most important representative of antifungal polyene macrolide antibiotics. However, it exhibits a number of undesirable properties associated mainly with severe toxicity and poor solubility. In the recent years many attempts have been made to obtain less toxic and water soluble derivatives of this antibiotic. Until now, amphotericin B was modified only at the polar head fragment of the molecule, namely at the amino and carboxyl groups[1,2]. Considering the contribution of other antibiotic moieties to its biological activity, we focused our interest on the cyclic, six-membered hemiketal formed from a ketone group at C-13 and the hydroxyl group at C-17[3].

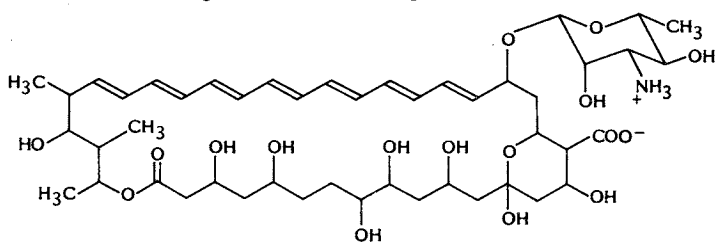
It has been shown that the amphotericin B molecule is rigid, stretched and rod-like[3]. In such a molecule the heptaene chromophore is well exposed to the van der Waals interaction with membrane sterols thus allowing the formation of a polyene-sterol complex permeabilizing the membrane[4,5]. We thought it to be of interest to see the biological effect of amphotericin B hemiketal ring cleavage that might influence the shape of the molecule and in consequence the antibiotic—target interaction. For this purpose we synthesized amphotericin B *O*-methyl oxime. The antibiotic is a good model for such a synthesis since unlike in a number of other polyenes, *e.g.* candidin[6], partricins[7], levorin A_2[8], it does not contain any free ketone group.

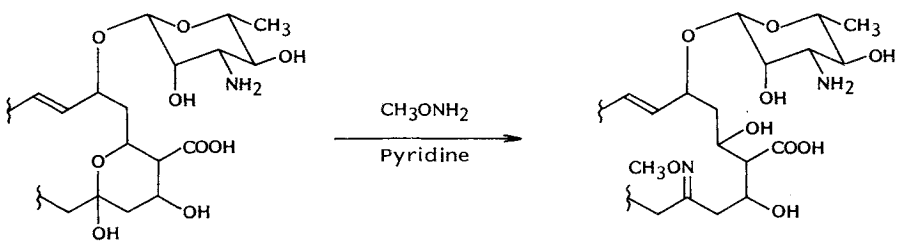
Materials and Methods

Amphotericin B was obtained from Hoffmann-La Roche and Co. (Basel, Switzerland).

The reaction was monitored by TLC on Silica gel 60 plates (Merck). UV spectra were determined with a Beckman Model 3600 spectrophotometer in MeOH. Circular dichroism measurements were done on a

Fig. 1. Structure of amphotericin B.





Jobin-Yvonne Mark V dichrograph in H_2O-EtOH (4:1) with sterol and polyene ratio of up to 20:1. ^1H and ^{13}C NMR spectra were obtained on a Varian VXR 300 spectrometer at 300 and 75 MHz, respectively. Spectra were recorded in DMSO-d_6 using solvent as an internal standard. FAB-MS was carried out on a VG 70-250 S mass spectrometer fitted with a saddle field FAB gun operated with xenon at 8 keV. 3-Nitrobenzyl alcohol was employed as the matrix.

Table 1. Rf values of amphotericin B and its *O*-methyl oxime on silica gel.

Compound	A	B	C
Amphotericin B	0.28	0.21	0.56
Amphotericin B *O*-methyl oxime	0.20	0.15	0.50

A: $CHCl_3$-MeOH-water (10:6:1).
B: EtOAc-AcOH-water (4:1:1).
C: 1-Butanol-pyridine-water (3:2:1).

Biological activity of amphotericin B and its derivative was determined as described previously[9].

Amphotericin B *O*-methyl oxime was obtained by the reaction indicated in Scheme 1.

The antibiotic derivative was prepared as follows: To a suspension of amphotericin B (0.277 g, 0.3 mmol) in pyridine (7.5 ml) methoxylamine hydrochloride (0.138 g, 1.65 mmol) was added under stirring at room temperature. After 25 hours, the reaction mixture was poured into a mixture of butanol (300 ml) and water (150 ml). The layers were separated and the organic layer was washed with water (3×50 ml) and concentrated *in vacuo*. After addition of ethyl ether (200 ml) the precipitate formed was separated by centrifugation, washed with ether and dried *in vacuo* to yield 0.183 g (64%) of the desired amphotericin B derivative. Chromatographic properties of the product and the parent antibiotic are given in Table 1. The derivative exhibited UV absorption maxima at the same wavelengths as amphotericin B. Examination of the product using positive ion FAB-MS (*m/z* 953, $(M+H)^+$) confirmed the expected molecular mass. Moreover, in the spectrum the abundant ion at *m/z* 790 ($(MH-\text{aminosugar})^+$) was observed indicating the presence of unchanged mycosamine and modified aglycone in the derivative examined.

The amphotericin B molecule offers two points of attack during the reaction with methoxylamine, namely hemiketal and lactone moieties. To evidence the presence of intact lactone we employed ^1H as well as ^{13}C NMR spectrooscopy. Thus, the ^1H NMR spectrum of the derivative showed a multiplet at 5.11 ppm attributable to the characteristic acyloxycarbinyl 37-H proton. The corresponding signal in the amphotericin B spectrum was observed at 5.19 ppm[10]. The ^{13}C NMR spectrum of amphotericin B *O*-methyl oxime contained signals at 170.84 and 170.74 ppm, assignable to the *syn* and *anti*-stereoisomers, in the same region as the lactone carbon absorption of the parent antibiotic (170.6 ppm[11]). The presence of the *O*-methyl oxime moiety was confirmed by the following signals: δ 157.89, 157.19 (-*C*=N-OCH_3, *syn*/*anti*-stereoisomers); δ 60.73 (-C=N-OCH_3). In the ^1H NMR spectrum the chemical shift of the *O*-methyl oxime methyl group was at 3.70 and 3.69 ppm (*syn*/*anti*-stereoisomers in a ratio of approximately 1:1).

Results and Discussion

The biological activity of the amphotericin B derivative was studied in comparison with that of the parent antibiotic and the results are summarized in Table 2. The activity of both compounds was examined towards ergosterol and cholesterol containing organisms, represented by the fungal cells and human erythrocytes, respectively. The data obtained provide evidence that the hemiketal ring cleavage in the antibiotic derivative led to a drastic decrease of its biological activity. This might be explained by the loss of the ability to interact with the membrane sterol targets and points to the essential role of the hemiketal

Table 2. Biological properties of amphotericin B and its *O*-methyl oxime.

Compound	MIC (μg/ml)		EH_{50}	EK_{50}
	Saccharomyces cerevisiae	*Candida albicans*	μg/ml	
Amphotericin B	0.15	0.18	1.7	0.34
Amphotericin B *O*-methyl oxime	3.20	2.75	>200	>100

EH_{50}: Concentration of compound which induced 50% hemolysis of human erythrocytes.
EK_{50}: Concentration of compound causing 50% potassium release from human erythrocytes.

moiety in the interaction considered. In fact, as evidenced by circular dichroism and UV spectroscopy, no polyene-sterol complex was formed.

As shown by the ^{13}C NMR studies[11~13], the presence of the hemiketal ring seems to be a common structural feature in the large-ring polyene macrolide antibiotics. We suppose that the essential contribution of this moiety to the biological activity is not restricted to amphotericin B but concerns also the other representatives of the large-ring group of polyene macrolides.

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