## Communications to the editor

# POLYENE MACROLIDE DERIVATIVES. I

## N-ACYLATION AND ESTERIFICATION REACTIONS WITH AMPHOTERICIN B

Sir:

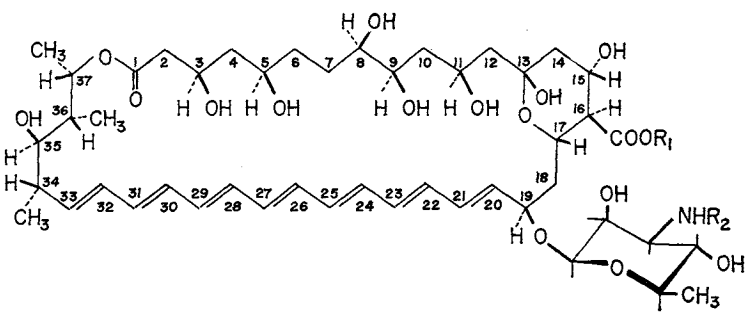
The total structure of the polyene antibiotic amphotericin B (Fig. 1) was recently determined in our laboratory by X-ray single crystal analysis[1]. In the course of preparation of suitable crystals for the X-ray studies a number of interesting derivatives were synthesized. We report here our approach to the synthesis of N-acyl and methyl ester derivatives and the effect of chemical modifications of amphotericin B on the retention of antifungal activity.

Amphotericin B and other polyene antibiotics[2] exhibit very poor solubility in water and common organic solvents with the exception of DMF* and DMSO**. This limited solubility appears to be predominantly due to the large molecular weight (700 to about 1,250) and the zwitterionic character of most of these antibiotics. The exceptional solubility in DMF and especially in DMSO must be related to a very strong solvent-solute interaction. Our IR studies of amphotericin B in DMSO solution revealed the presence of strong intermolecular hydrogen bonds involving not only the hydroxyl groups but the carboxyl and the amino groups as well. The IR spectrum also suggested the disruption of the zwitterionic character of the antibiotic by molecules of solvent, thus facilitating the exposure of both functional groups to chemical reactions.

N-Acylation of amphotericin B (Fig. 1) and some other polyene antibiotics has been reported previously[3]. It involved the treatment of the antibiotics with a very large excess (50 moles) of acyl anhydride at room temperature for 1 hour. The yield of a crude product was 70~75%. After DMSO was employed in our N-acylation procedure a small excess (0.1~1.0 mole) of acyl anhydride was sufficient to complete the reaction in 10~30 minutes at ice bath temperature with a yield better than 90%. In a typical example, 1 g of amphotericin B (97% pure) was dissolved with stirring in 10 ml of DMSO at room temperature and diluted with 10 ml of absolute methanol. The mixture containing partially precipitated antibiotic was cooled in ice and 0.12 g (0.15 mol excess) of acetic anhydride was added stepwise during 10 minutes while stirring. After allowing 10 minutes for completion of the reaction, the product was isolated by precipitation in 100 ml of anhydrous ethyl ether, followed by centrifugation and wash-

Fig. 1. The structure of amphotericin B and its derivatives

I. Amphotericin B; $R_1=H$; $R_2=H$
II. Amphotericin B methyl ester; $R_1=CH_3$; $R_2=H$
III. N-Acyl amphotericin B; $R_1=H$; $R_2=acyl$
IV. N-Acyl amphotericin B methyl ester; $R_1=CH_3$; $R_2=acyl$



* N,N-Dimethylformamide.

** Methyl sulfoxide (dimethyl sulfoxide).

Table 1. Derivatives of amphotericin B and their antifungal activity

| Amphotericin B and derivatives | Formula of functional groups involved | | Minimal inhibitory concentration ($\mu$g/ml) | | |
|---|---|---|---|---|---|
| | Carboxylic | Amino | *Saccharomyces cerevisiae* #216 | *Aspergillus niger* #1 | *Candida albicans* #204 |
| Amphotericin B | $-COO^-$ | $-NH_3^+$ | 0.05～0.5 | 0.5 ～1.0 | 0.05～0.5 |
| N-Acetylamphotericin B | -COOH | $-NH\cdot CO\cdot CH_3$ | 1.0 ～10.0 | 1.0 ～10.0 | 1.0 ～10.0 |
| N-Iodoacetylamphotericin B* | -COOH | $-NH\cdot CO\cdot CH_2I$ | 0.5 ～1.0 | 1.0 ～10.0 | 0.5 ～1.0 |
| N-Propionylamphotericin B | -COOH | $-NH\cdot CO\cdot C_2H_5$ | 1.0 ～10.0 | 1.0 ～10.0 | 1.0 ～10.0 |
| N-Succinylamphotericin B | -COOH | $-NH\cdot CO\cdot C_2H_4\cdot COOH$ | 1.0 ～10.0 | 1.0 ～10.0 | 1.0 ～10.0 |
| N-Phthalylamphotericin B | -COOH | $-NH\cdot CO\cdot C_6H_4\cdot COOH$ | 1.0 ～10.0 | 1.0 ～10.0 | 1.0 ～10.0 |
| Amphotericin B methyl ester | $-COOCH_3$ | $-NH_2$ | 0.05～0.5 | 0.05～0.5 | 0.05～0.5 |
| N-Acetylamphotericin B methyl ester | $-COOCH_3$ | $-NH\cdot CO\cdot CH_3$ | 1.0 ～10.0 | 0.5 ～1.0 | 0.5 ～1.0 |
| N-Iodoacetylamphotericin B methyl ester | $-COOCH_3$ | $-NH\cdot CO\cdot CH_2I$ | 1.0 ～10.0 | 1.0 ～10.0 | 0.5 ～1.0 |
| N-Propionylamphotericin B methyl ester | $-COOCH_3$ | $-NH\cdot CO\cdot C_2H_5$ | 1.0 ～10.0 | 0.5 ～1.0 | 0.5 ～1.0 |
| N-Succinylamphotericin B methyl ester | $-COOCH_3$ | $-NH\cdot CO\cdot C_2H_4\cdot COOCH_3$ | 1.0 ～10.0 | 0.5 ～1.0 | 0.5 ～1.0 |
| N-Phthalylamphotericin B methyl ester | $-COOCH_3$ | $-NH\cdot CO\cdot C_6H_4\cdot COOCH_3$ | 10.0 ～30.0 | 0.5 ～1.0 | 1.0 ～10.0 |

* Crystalline material used in X-ray studies[1].

ing with ethyl ether. After drying *in vacuo* the yield was 95 %, and the purity was 96 % as measured by spectrophotometric extinction. A single spot was obtained on TLC*. The N-acylation was confirmed by quantitative ninhydrin assay and by consideration of IR and NMR (in DMSO-$d_6$) spectra.

Esterification of the carboxylic group in amphotericin B (Fig. 1) or other polyenes with accompanying retention of antifungal activity, is not described in the available literature. Realizing the difficulties involved in such an esterification, an attempt was made to employ DMSO as a reaction medium. Amphotericin B was dissolved in this solvent and reacted with diazomethane prepared in THF**. Tetrahydrofurane was used instead of ethyl ether to avoid precipitation of the antibiotic. In a typical synthesis, 1 g of amphotericin B (97 % pure) was dissolved at room temperature in 10 ml of DMSO and diluted with 1 ml of absolute methanol***. After cooling in ice to +6°C the solution was treated with about 7 ml of diazomethane reagent during a 1 minute period. The esterification was complete at that time, and the product was isolated by precipitating in 200 ml of anhydrous ethyl ether followed by centrifugation and ether washing. The yield after drying *in vacuo* was 90 % and the purity 95 % as measured by extinction. TLC**** revealed one major spot and a very smalll amount of some by-products different in Rf value from the parent antibiotic. The esterification of the carboxylic group was confirmed by quantitative titration, IR and NMR (in DMSO-$d_6$) studies. With N-acyl derivatives of amphotericin B used as starting materials for esterification a number of N-acyl amphotericin B methyl esters were also synthesized. The progress of the above described syntheses and the end points of reactions were monitored by thin-layer chromatography.

Table 1 summarizes the prepared derivatives of amphotericin B and illustrates the degree of retention of antifungal activity. The most significant fact given in Table 1 is the full retention of antifungal activity of the methyl ester of amphotericin B. N-

* TLC on Silica gel G plate 20×20 ; system : chloroform - methanol - 0.025 M borate buffer, pH 8.3 (2 : 2 : 1 v/v, lower phase).

** Tetrahydrofurane. The reagent was prepared in the usual way from Diazald (R) (N-methyl-N-nitroso-*p*-toluenesulfonamide) only the ethyl ether was replaced by THF, free of peroxides. The resultant concentratiion of diazomethane in THF solution was 0.3～0.4 molar.

*** To minimize side reactions.

**** The same system as employed for N-acyl derivatives.

Acylation of amphotericin B drastically reduces the activity. On the other hand, esterification of the N-acyl derivatives did not change their residual activity. These facts indicate that a free amino group is necessary for the retention of the full antifungal activity. In contrast, esterification of the carboxyl group has little effect on the activity of the antibiotic.

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