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Chemical Modification of Tetraene Macrolide Antibiotic Nystatin A_1 with Organophosphorus Alcohols

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Abstract—Esterification of tetraene macrolide antibiotic nystatin A_1 with organophosphorus alcohols has been studied; formation of the corresponding organophosphorus esters of the antibiotic has been confirmed. Physicochemical, spectral, and biological properties of the prepared esters have been studied. The esterification products have revealed high antifungal activity towards the *Candida* yeast fungi.

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Nystatin was the first polyene macrolide antibiotic discovered and introduced into medical practice. It has been used over more than 50 years for treatment of many clinical forms of candidosis caused by the *Candida* yeast fungi [1–3]. However, efficiency of this antifungal antibiotic is significantly limited because of its high toxicity, poor solubility in water and due to reduced sensitivity of fungal microorganisms towards its action [4–6]. In view of that, development of new nystatin derivatives with improved medical and biological properties has been in progress.

A variety of semi-synthetic derivatives of nystatin A_1 as well as its water-soluble salts and their complexes are known [7–9]. Previously we prepared the hydrophosphoryl [10], fluorinated [11], and organosilicon [12] derivatives of nystatin A_1. Extending those studies on nystatin A_1 chemical modification, here we report on its reactions with organophosphorus alcohols. Esters of polyene macrolide antibiotics obtained via esterification with aliphatic alcohols were the first semi-synthetic derivatives of those antifungal drugs [7–9]. To the best of own knowledge, organophosphorus alcohols were not previously used for modification of macrolide antibiotics.

We demonstrated the formation of the organophosphorus esters **I** and **II** in reactions of nystatin A_1 with organophosphorus alcohols (see Scheme 1).

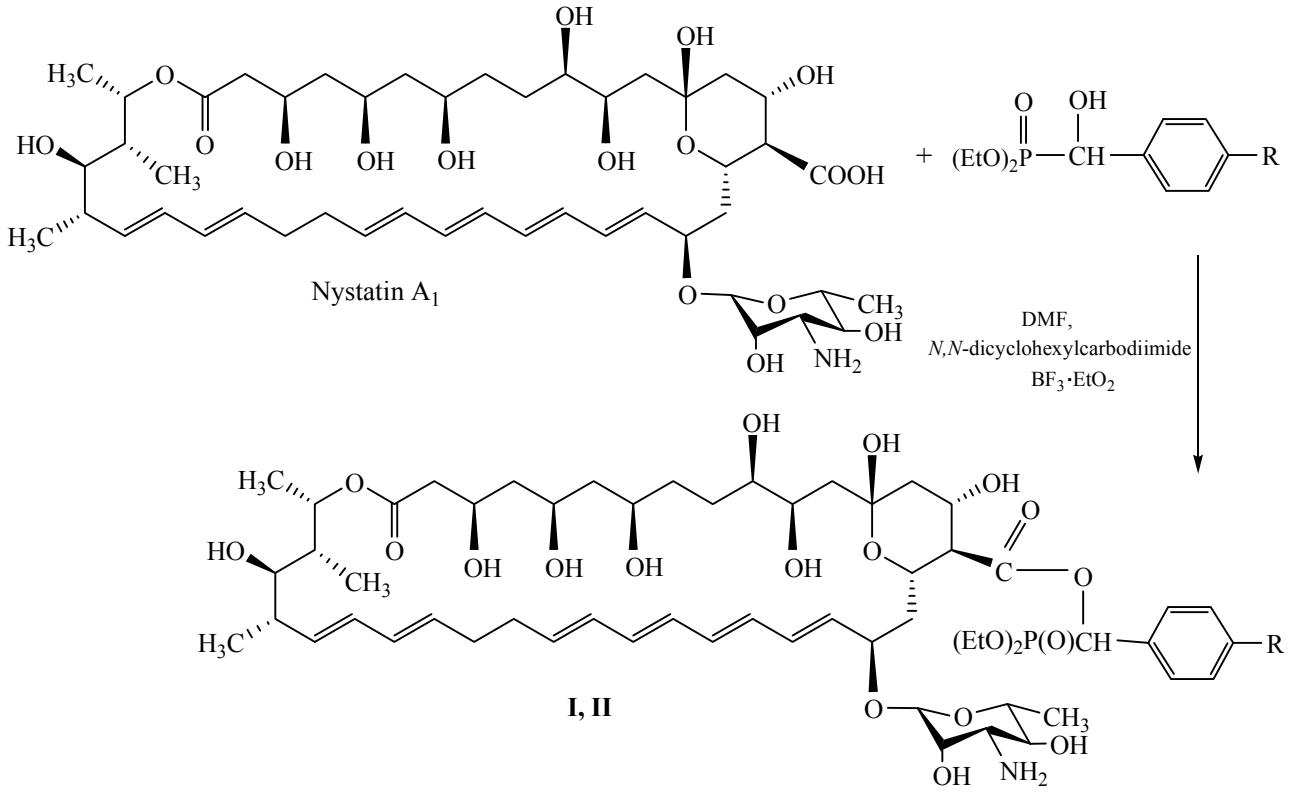
The following organophosphorus alcohols were used: diethyl[hydroxy-(4-methoxyphenyl)methyl]phosphonate and diethyl[hydroxy-(4-chlorophenyl)methyl] phosphonate. The studied reactions of nystatin A_1 with the alcohols occurred in anhydrous dimethylformamide (DMF) in the presence of *N,N*-dicyclohexylcarbodiimide and catalytic amount of boron trifluoride etherate at 0–20°C. The reactions were performed during 10 h under argon stream at vigorous stirring. The reactions were in fact modified procedure to prepare esters of polyene macrolide antibiotics described in [13]. It was demonstrated that the chemical modification of nystatin A_1 with organophosphorus alcohols was highly selective to yield the respective organophosphorus esters. However, the yields of **I** and **II** were as low as 51% and 54%, respectively, evidently, due to lability of initial nystatin A_1 under reaction conditions and of the derivatives under conditions of isolation and chromatographic purification.

The prepared solid nystatin derivatives **I** and **II** did not reveal sharp melting point, being decomposed upon heating. They were readily soluble in dimethylsulfoxide and DMF; moderately soluble in methanol, ethanol, and pyridine; insoluble in water, acetone, chloroform, diethyl ether, benzene, and hexane.

Structures of derivatives **I** and **II** was confirmed by NMR (^{1}H, ^{13}C, and ^{31}P), IR, and UV spectroscopy. In their ^{1}H NMR spectra, the proton signals typical of

† Deceased.

Scheme 1.



R = OCH_3 (**I**), Cl (**II**).

nystatin A_1 [13, 14] were observed, ethoxy groups protons were assigned to triplet signals of CH_3 (1.21–1.23 ppm) and doublet of quartet signals of CH_2 (3.67–3.69 ppm). The phosphorus coupling constant J_{HP} was 11.2 Hz. Methine proton was assigned to doublet signal at 4.87–4.90 ppm, $^2J_{HP} \approx 9.7$ Hz. Protons of *para*-substituted phenyl ring gave rise to two pairs of chemically equal but magnetically unequal nuclei ABA^1B^1. ^{13}C spectra of **I** and **II** contained signals of nystatin A_1 [14–16] carbon atoms; the carbon atom adjacent to phosphorus was assigned to typical doublet at about 70 ppm with J_{CP} of about 160 Hz. $\underline{CH_3}CH_2O$ carbon atoms were assigned to the 16.9–17.0 ppm signals, $CH_3\underline{CH_2}O$ carbon atoms were assigned to the 63.1–63.7 ppm signals, and phenyl ring carbon atoms resonated at 127.0–136.0 ppm. Phosphorus chemical shifts were 22.3 ppm (**I**) and 21.7 ppm (**II**) that was typical of resonance of ^{31}P at the sp^2 carbon atom [17, 18].

IR spectra of the derivatives **I** and **II** contained, along with the bands typical of nystatin A_1 [16], the absorption bands at 1240–1245 and 1568–1574 cm^{-1} assigned to the phosphoryl group P=O and phenyl substituents, respectively. The electron absorption spectra of the derivatives contained the bands with maxima at 292, 304, and 319 nm, typical of the conjugated tetraene system [19].

As the initial antibiotic nystatin has been applied in candidosis treatment [2, 3], the biological tests were performed to estimate the antifungal activity of **I** and **II** towards a series of cultures of the *Candida* genus. In particular, the tested derivatives revealed high antifungal activity towards *Candida albicans*, *Candida utilis*, *Candida tropicalis*, *Candida krusei*, *Candida glabrata, Candida lusitaniae, Candida parapsilosis*, *Candida kefyr*, and *Candida guillermondii*, the minimal fungistatic concentration being of 1.56 to 25.0 μg/mL. Noteworthily, the respective concentration of the parent nystatin A_1 was ranged between 1.56 to 12.5 μg/mL.

To conclude, the suggested approach to prepare organophosphorus esters of nystatin A_1 is a promising direction of development of new semi-synthetic antifungal drugs.

EXPERIMENTAL

Nystatin from "Biosintez" (Russia) was used of biological activity 5000 U/mg, specific absorption

ε = 700 L mol^{-1} cm^{-1} at wavelength λ = 304 nm. Nystatin is known to be a complex drug containing the A_1, A_2, and A_3 tetraene components; the used batch contained more than 90% of nystatin A_1, less than 9% of nystatin A_2, and less than 1% of nystatin A_3 [9]. The major component was isolated by means of chromatography as described in [20, 21]. The organophosphorus alcohols were prepared following the method adopted from [22, 23]. Organic solvents were purified as described in [24].

NMR spectra (^1H, ^{13}C, COSY, DEPT, and HMQC) were registered with Bruker Avance spectrometer (500 MHz, DMSO-d_6 as solvent, TMS as internal reference). IR spectra were recorded with Bruker Vector 22 spectrometer (KBr). UV spectra were obtained with Ultrospec 2100pro spectrophotometer (Biochrom). Purity of the derivatives **I** and **II** was confirmed by TLC (Silica Gel 60 F_{254} plates, 0.25 mm, Merck) with eluents: A, $CHCl_3$–MeOH–*n*-propanol–borate buffer (pH 8.14), 3 : 2 : 2 : 1; and B, MeOH–acetone–AcOH, 8 : 1 : 1. The plates were developed with UV. Silica Gel 60 (63–200 μm, Merck) was used as sorbent.

Nystatin A_1 was used as reference in all biological tests. Antifungal activity was determined by serial dilutions in liquid nutrient solution (two-fold dilution at each step). The minimal fungistatic concentration was evaluated by visual inspection of the culture growth intensity in the testing and reference specimens. The experiments were performed in triplicate.

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