METHYL ESTERS OF TRIMETHYL-AMMONIUM DERIVATIVES OF POLYENE MACROLIDE ANTIBIOTICS

Sir:

A number of derivatives of polyene macrolide antibiotics had been synthesized in order to improve their water-solubility and reduce the toxicity[1~5].

We report the synthesis of a new group of derivatives of these antibiotics, namely methyl esters of N-methylated polyene macrolides, further indicated as DMS derivatives. These substances are prepared upon the treatment of an alcoholic solution of the antibiotic with dimethyl sulphate in the presence of a neutralizing agent. Under these conditions the amino and carboxylic groups are methylated. In the course of the reaction the aliphatic amino group forms trimethylammonium salts, and subsequently the carboxylic group is esterified. The aromatic amine, present in the molecules of some heptaenes, is also partially methylated yielding mono- and dimethyl derivatives.

Optimal conditions of reaction are the following: A mixture of N,N-dimethylacetamide and methanol as solvent, temperature range of 25~35°C, 10 molar excess of dimethyl sulphate and sodium hydrogen carbonate. The course of the reaction can be followed by thin-layer chromatography on silica gel in the solvent system, chloroform - methanol - water, (20: 10: 1, v/v). The reaction is completed within 4~24 hours, depending on the polyene macrolide used. After removal of undissolved sodium hydrogen carbonate the products are precipitated with ethyl ether, the precipitates are dissolved in butanol saturated with water, and the resultant solution is washed with water and concentrated under diminished pressure. The crude derivatives are again precipitated with ethyl ether and the precipitates are washed with ethyl ether and dried in vacuum. The yields are 70~80% depending on the antibiotic used. The derivatives were purified by counter-current distribution with the solvent system, chloroform - methanol - water, (2: 2: 1, v/v) or by partition chromatography on silica gel or Sephadex LH-20 with chloroform - methanol - water, (20: 10: 1, v/v).

Derivatives of polyene macrolides representing major structural groups (pimaricin, nystatin, amphotericin B, perimycin, mycoheptin, candicidin, levorin, trichomycin and aureofacin) were synthesized and characterized.

In a typical synthesis, to 1 g of candicidin ($E^{1\%}_{1cm}$ 800 at 378 nm) dissolved in 20 ml of N,N-dimethylacetamide - methanol solvent mixture (20: 1, v/v), 1 g of sodium hydrogen carbonate and 1 ml of dimethyl sulphate were added portionwise, and the reaction mixture was stirred for 24 hours at 25°C under nitrogen in darkness. The undissolved sodium hydrogen carbonate removed by centrifugation, the methanol component was evaporated under diminished pressure, and the derivative was precipitated by the addition of 300 ml of ethyl ether. The product was dissolved in 70 ml of water-saturated butanol and the butanol layer was washed two times with 20 ml of water and concentrated under diminished pressure.

Precipitation with ethyl ether followed by washing with ethyl ether and hexane and drying in vacuum yielded 0.8 g of crude derivative ($E^{1\%}_{1cm}$ 830 at 378 nm). The derivative was further purified by means of counter-current distribution using the solvent system chloroform - methanol - water (2: 2: 1, v/v), and applying 120 transfers. This afforded 0.4 g of DMS-candicidin. The derivative exhibited electronic absorption maxima of the same wavelength as candicidin itself at λ 359, 378 ($E^{1\%}_{1cm}$ 900), 401 nm. The oscillation bands at $\nu = 1730\,cm^{-1}$ (–C–O–OCH_3), lack of $1590\,cm^{-1}$ (–C–O–O^-) revealed in the IR spectrum and peak at δ ($CDCl_3$) = 3.65 (–C–O–O–CH_3) in the ^1H NMR spectrum of persilylated DMS-candicidin documented the presence of methylated carboxylic group.

Hydrolysis of DMS-candicidin in acidic conditions afforded N,N,N-trimethylammonium salt of mycosamine (FD-mass spectrum—m/e = 206 (MW); ^1H NMR—δ (D_2O) = 3.68 ($(CH_3)_3$–N–). Treatment of DMS-candicidin with 4% sodium hydroxide solution yielded N,N-dimethyl-, N-methyl- and unsubstituted *p*-aminoacetophenone in molar ratios 3: 5: 2, respectively.

In another typical synthesis the treatment of 2 g of amphotericin B ($E^{1\%}_{1cm}$ 1400 at 382 nm) with dimethyl sulphate under identical conditions, using the same molar proportions of reagents, yielded 1.7 g of crude methyl ester of N,N, N-trimethylamphotericin B methyl sulphate ($E^{1\%}_{1cm}$ 1200 at 382 nm).

The antifungal and hemolytic activities of these

Table 1. Antifungal and hemolytic activities of polyene macrolide antibiotics and their DMS-derivatives.

Antibiotic	IC_{50}* (mcg/ml)	EH_{50}** (mcg/ml)
Pimaricin	1	100
DMS-pimaricin	1.7	400
Polyfungin	0.08	20
DMS-polyfungin	0.15	120
Nystatin	0.1	50
DMS-nystatin	0.25	100
Amphotericin B	0.03	5
DMS-amphotericin B	0.05	15
Candicidin	0.005	2.5
DMS-candicidin	0.003	15
Aureofacin	0.005	0.35
DMS-aureofacin	0.003	15

* The concentration of the substance causing 50% inhibition of the growth of *Saccharomyces cerevisiae* in SABOURAUD liquid medium determined photometrically (λ 660 nm) after 24-hour incubation at 28°C.

** The concentration of the substance causing 50% release of hemoglobin from human erythrocytes (1 ml in 250 ml of isotonic NaCl solution) determined photometrically at λ 550 nm.

synthetic derivatives are presented in Table 1. The concentrations of the test substances inhibiting 50% of the growth of *Saccharomyces cerevisiae*, and the concentrations producing 50% release of hemoglobin from erythrocytes were taken as measures of the antifungal and hemolytic activities respectively.

The DMS derivatives of polyene macrolides display antifungal activity of the same range or up to 2.5 times lower as compared to the parent antibiotics and parallel substantially lower hemolytic activity. Similar advantageous ratio of EH_{50}/IC_{50} is observed for methyl ester of polyene macrolides.

The compounds obtained, being quarternary ammonium salts, are readily soluble in water.

The high biological activity of these compounds along with their excellent water solubility indicate that DMS-derivatives of polyene macrolides are encouraging novel antifungal agents.

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