# POLYENE ANTIBIOTICS. IX

## AN IMPROVED METHOD FOR THE PREPARATION OF METHYL ESTERS OF POLYENE ANTIBIOTICS[1]

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An improved general method for the preparation of methyl esters of polyene antibiotics is discussed. Using this method methyl esters of pimaricin, nystatin, eurocidin, hamycin, hamycins A and B, aureofungin, partricins A and B, candimycin, candicidin, and amphotericin B have been prepared and their physical properties are reported. The biological activities of hamycins A and B and their methyl esters are also described.

Polyene antibiotics continue to be of interest for their diverse biological activities. Amphotericin B is the drug of choice in treating systemic human fungal infection, nystatin is widely employed for topical fungal treatment, and at least one polyene antibiotic, candicidin, has been reported to reduce benign prostatic hyperplasia[2] and to lower blood cholesterol.[3] Recently it was shown[4~11] that the methyl esters of polyene antibiotics, at least of heptaene antibiotics, show improvement in toxicity and therapeutic ratio relative to those of the parent compound. In consequence, the methyl esters have acquired a potentially important role in the clinical applications of polyene antibiotics. The method previously described for the preparation of methyl esters of polyene antibiotics involved the use of diazomethane and dimethyl sulfoxide[5,12] solvent; other solvents recommended[13] were dimethylformamide, dimethylacetamide, formamide, ethyl-Cellosolve and pyridine. These solvents have the ability to dissolve the antibiotic, but they are relatively expensive, their high boiling points make them difficult to remove after the reaction, and they tend to decompose the product.

We have now devised an improved method for the preparation of methyl esters of polyene antibiotics, which does not involve the use of dimethyl sulfoxide or the other reported solvents. Our new procedure involves the use of a suspension of the antibiotic in methanol instead of a solution in one of the other solvents. A solution of diazomethane in tetrahydrofuran (prepared by the standard procedure)[8,14] is then added to this stirred suspension and the mixture is stirred at room temperature; as the esterification proceeds, the antibiotic goes into solution. After solution a slight excess of diazomethane is added and the mixture is further stirred at room temperature for $1 \sim 2$ hours before excess diazomethane is destroyed by a few drops of acetic acid. The methyl ester formed is soluble in this solvent mixture, but, if the starting material is impure, some insoluble material may remain and can be filtered after decomposition of the excess diazomethane. This provides, of course, a partial purification of the methyl ester. Most of the solvent is then removed under vacuum below 40°C and the product is precipitated by addition of ether.* The precipitate is centrifuged or filtered, washed with fresh ether,

* Since the solubility of the methyl ester is greater in the previously employed ether-dimethyl sulfoxide, a far greater excess of ether is required to precipitate the methyl ester from dimethyl sulfoxide solution than is required in the present method.

Fig. 1. $^1$H NMR spectrum of pimaricin methyl ester.
The shaded peaks disappear on shaking with deuterium oxide.

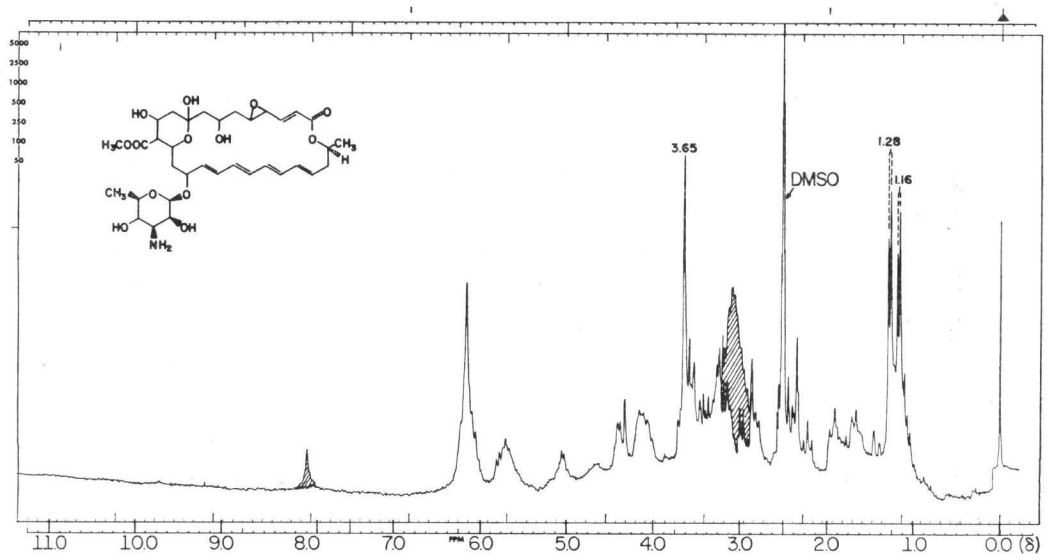
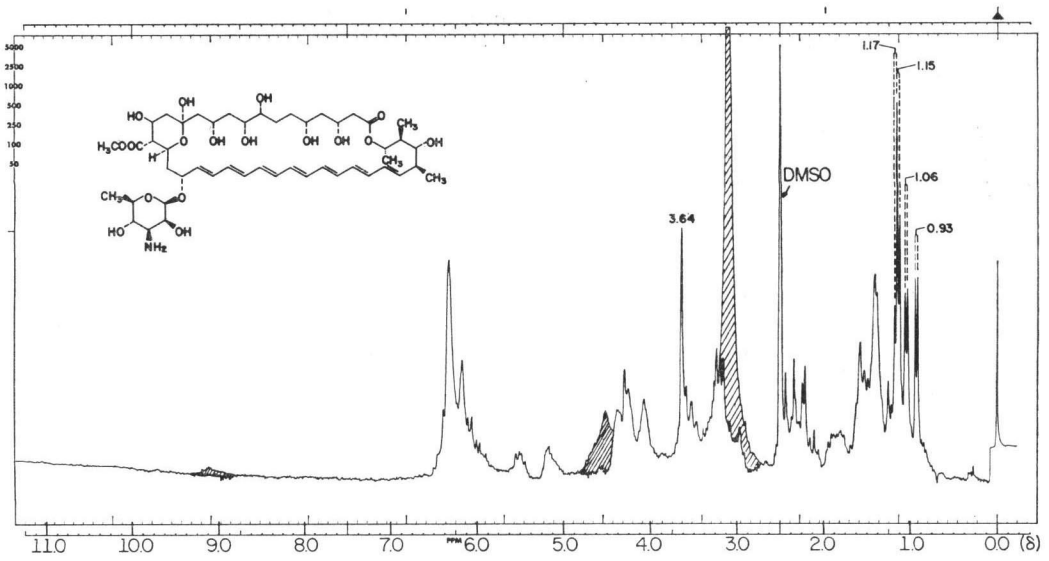



Fig. 2. $^1$H NMR spectrum of amphotericin B methyl ester.
The shaded peaks disappear on shaking with deuterium oxide.




and dried under high vacuum.* Recoveries of methyl esters employing the present procedure range from 80 to 100% and are usually nearly quantitative. The products are essentially pure as judged by their tlc behavior. Purity of the methyl esters, as judged by ultraviolet extinction coefficients, depends also, however, on the purity of the starting polyene antibiotics. Thus, a sample of hamycin with $E^{1\%}_{1cm}$ 585 at 379 nm gave a methyl ester with $E^{1\%}_{1cm}$ 586 at 379 nm, while the corresponding value for the methyl

* In the earlier methods, it was necessary to reprecipitate the product to remove traces of dimethyl sulfoxide. With the lower boiling methanol (and tetrahydrofuran) this is not required.

ester from a second sample of hamycin ($E^{1\%}_{1cm}$ 742 at 379 nm) was 774. Melting points were not a reliable indication of purity. We shall describe $^{1}H$ and $^{13}C$ NMR spectra in detail elsewhere; however, Figs. 1 and 2 show the $^{1}H$ NMR spectra of the methyl esters of pimaricin and amphotericin B, two structurally known tetraene and heptaene antibiotics.

As noted above, biological properties of a few of the polyene antibiotics' methyl esters have been described previously.[4~11] These include nystatin, pimaricin, amphotericin B, candicidin, and candimycin, which we have prepared here also. The antifungal and antiprotozoal properties of hamycins A and B, components separated by countercurrent distribution of hamycin complex, and their methyl esters are shown in Table 1. From the data presented there it is clear that the methyl esters remain active antifungal and antiprotozoal agents, though their minimum inhibitory concentrations are somewhat greater than those of their parent antibiotics. More importantly, the toxic levels of the methyl esters are much higher. For example, ataxia was observed for hamycin A at 12.5 mg/kg (and death at 25 mg/kg) but for its methyl ester at 100 mg/kg (all by i.p. administration); for hamycin B death was observed at 100 mg/kg, while its methyl ester was essentially non-toxic (also by i.p. administration). Reduced toxicity is in accord with published results for other polyene antibiotic methyl esters.[4,5]

Table 1. *In vitro* antifungal and antiprotozoal activity of hamycins A and B and their methyl esters

| Test organisms | Minimal inhibitory concentration ($\mu$g/ml) | | | |
|---|---|---|---|---|
| | Hamycin A | Hamycin A methyl ester | Hamycin B | Hamycin B methyl ester |
| *Candida albicans*[a,b] | 0.015 | 0.25 | 0.5 | 1.0 |
| *Trichophyton mentagrophytes*[a,b] | 0.5 | 1.0 | >20 | >20 |
| *Trichomonas foetus*[a,b] | 1.25 | >10 | 2.5 | >10 |
| *Blastomyces dermatidis* UC 1466[c,d] | <1 | <10 | <1 | <1 |
| *Geotrichum* sp. UC 1207[c] | <1 | >100 | ≥1 | <10 |
| *Hormodendrum compactum* UC 1222[c] | ≥1 | ≥10 | ≥1 | ≥1 |
| *Phialophora verrucosa* UC 1807[c] | <1 | ≥10 | ≥1 | ≥1 |
| *Cryptococcus neoformans* UC 4869[c,d] | <1 | <10 | <1 | <1 |
| *Cryptococcus neoformans* UC 1139[c] | ≥10 | ≥100 | ≥10 | ≥100 |
| *Trichophyton rubrum* UC 1458[c] | >100 | ≥10 | <100 | <100 |
| *Trichophyton violaceum* UC 1459[c] | >100 | <10 | <100 | ≥10 |
| *Trichophyton asteroides* UC 4775[c] | ≥100 | >100 | <10 | <100 |
| *Trichophyton mentagrophytes* UC 4797[c] | <100 | >100 | <100 | <100 |

[a] By tube dilution technique (Dr. M. H. PINDELL, Panlabs, Inc.).
[b] Measured MIC for amphotericin B=0.25 $\mu$g/ml *vs. C. albicans* and *T. mentagrophytes*, 1.25 $\mu$g/ml *vs. T. foetus*; measured MIC for metronidazole=0.5 $\mu$g/ml *vs. T. foetus*.
[c] By agar dilution technique, tested as suspensions (Dr. J.J. VAVRA, Mr. G.E. ZURENKO, The Upjohn Co.).
[d] Animal strain.

## Experimental

Melting points, determined on a Kofler hot stage apparatus, are uncorrected. Infrared spectra were taken on a Beckman IR12 spectrophotometer. Unless otherwise stated, ultraviolet spectra were determined in methanol containing 4% dimethylformamide on a Beckman spectrophotometer, Model Acta MVI. Optical rotations were measured on a Zeiss polarimeter. Proton magnetic resonance ($^{1}H$ NMR) spectra were determined in DMSO-$d_6$ by Mr. M. L. MILLER on a Varian HR-220 spectrometer equipped with a Nicolet TT220 FOURIER transform accessory. Chemical shifts are reported as ppm relative to tetramethylsilane as internal standard. Hamycins A and B and partricins A and B were isolated by countercurrent distribution using methanol - chloroform - 0.05 M borate buffer (pH 8.3)

(2: 2: 1) as the solvent system.[15] Analytical thin-layer chromatography was carried out on Analtech precoated (250 $\mu$) silica gel G plates using 1-butanol - ethanol - acetone - 30% ammonium hydroxide (2: 5: 1: 3, v/v) as the developing solvent.[5] The spots were visualized either by iodine vapors, UV light or sulfuric acid spray.

Standard Esterification Procedure.

In the standard procedure a suspension of 5 g of the antibiotic in 100 ml of absolute methanol was stirred while a 0.10 to 0.15 molar solution of diazomethane (determined by esterifying a known quantity of acetic acid) in *ca.* 100 ml of tetrahydrofuran was added slowly, so that the reaction did not become too vigorous, then at room temperature for 2 hours. As the esterification proceeded, the methyl ester went into solution during the first 5~15 minutes. Excess diazomethane was destroyed by adding a few drops of acetic acid until effervescence ceased, and the solution was filtered if necessary. Any solid collected was washed with a little fresh methanol. The filtrate was concentrated to *ca.* 40 ml and the product was precipitated by addition of *ca.* 200 ml of ether, centrifuged, washed with fresh ether (3× 100 ml) and dried. A second crop was obtained in some cases by addition of more ether. The physical properties of some of the methyl esters prepared by this method are given in Table 2.

Table 2. Yields and physical properties of methyl esters[a] of polyene antibiotics.

| Antibiotics | Methyl esters[a] | | | | | |
|---|---|---|---|---|---|---|
| | Yield (%) | Mp (°C, dec) | Rf | $[\alpha]_D^{26}$ DMF (°) | $\lambda_{max}^{MeOH}$ ($E_{1cm}^{1\%}$) | $\nu_{KBr}$ ($cm^{-1}$) |
| Pimaricin[b] | 83 | 270~280 | 0.70 | +94.0 | 304 (719) | 1722 |
| Nystatin[b] (Lederle) | 96 | 110~116 | 0.71 | +34.0 | 304 (615) | 1730 |
| Nystatin[b] (Squibb) | 100 | 110~115 | 0.71 | +45.3 | 304 (697) | 1730 |
| Eurocidin[b] | 75 | 150~160 | 0.77[e] | +121.2 | 350 (869) | 1728 |
| Hamycin[b] | 89 | 155~160 | 0.80[e] | +98.7 | 379 (774) | 1725 |
| Hamycin A[c] | 92 | 145~155 | 0.80 | +125.4 | 379 (843) | 1725 |
| Hamycin B[c] | 93 | 144~154 | 0.81 | +88.0 | 379 (727) | 1725 |
| Aureofungin[b,d] | 80 | 155~160 | 0.82[e] | +60.9 | 378 (576) | 1732 |
| Partricin A[c,d] | 95 | 154~158 | 0.82 | +47.6 | 377 (811) | 1728 |
| Partricin B[c,d] | 99 | 145~149 | 0.84 | +74.2 | 379 (841) | 1728 |
| Candimycin[b] | 90 | 140~145 | 0.84[e] | +145.9 | 377 (725) | 1728 |
| Candicidin[b] | 100 | 140~145 | 0.83[e] | +86.1 | 378 (618) | 1720 |
| Amphotericin B[b] | 100 | 200~210 | 0.79 | +365.8 | 382 (1440) | 1725 |

[a] Prepared by the method described in the text.
[b] Commercial samples.
[c] Purified samples.
[d] The biological properties of these and their water soluble salts will be described in a subsequent report.
[e] Major spot.

When methanol was replaced by ethanol, 2-propanol or dioxane in the procedure, the recoveries were reduced to 2/3, 1/2, and 1/3, respectively, but the purity of the product was somewhat better as judged by UV spectroscopy. The unreacted insoluble material was separated by filtration before working up the product.

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