Communications to the editor

NEW ANTITUMOR ANTIBIOTICS: MUSETTAMYCIN AND MARCELLOMYCIN FROM BOHEMIC ACID COMPLEX

Sir:

As a result of screening cultures of actinomycetes for metabolites having antitumor properties, we have isolated an anthracycline mixture, bohemic acid complex, from fermentations of *Actinosporangium* sp. strain C-36,145 (ATCC 31127). Subsequently, two pure anthracyclines, musettamycin and marcellomycin, both glycosides of ε-pyrromycinone, were obtained by fractionation of the complex.

Bohemic acid complex was produced by shake flask cultures from a seed culture grown for 6 days at 27°C on agar slant medium containing 2% oatmeal, 0.2% D-glucose, 0.2% soy peptone, and 0.2% agar. Actual production was in two stages from spores, first a vegetative culture at 27°C for 48 hours in medium containing 3% D-glucose, 1% soy bean flour, 1% Pharmamedia (Traders Oil Mill Co., Fort Worth, Texas), and 0.3% $CaCO_3$ and finally a production culture at 27°C for 144 hours in a medium containing 5% glycerol, 2% soybean flour, 1% peanut meal, and 1% $CaCO_3$. Stir-jar and tank fermentations were carried out in the same production medium after inoculation with vegetative culture. Productivity was measured by antibiotic plate assays *vs. Bacillus subtilis* (pH 6) and *in vivo vs.* the L-1210 tumor system in the mouse.

Extraction of the whole broth at its harvest pH 8.1 with methyl *iso*-butyl ketone, concentration of the organic phase to remove as much solvent as possible, and dilution with petroleum ether gave bohemic acid complex as a dark red amorphous solid. In the larger scale extractions the last was ether washed to remove oily contaminants. Initial fractionation of the complex was carried out on Sephadex LH-20 using chloroform as the eluting solvent, cuts being followed spectrophotometrically at 490 nm. Four distinct bands of anthracycline pigments eluted and, after work-up, were evaluated by thin-layer chromatography on Brinkmann 60F24 silica gel plates using an 8:2 toluene - methanol system. The first band, eluting at the front, was a complex inactive mixture, whereas the other three bands gave essentially single zones with Rf values respectively of 0.75, 0.3, and 0.3., the last moving slightly slower than the third. The second band material was crystalline and readily identified as the known inactive anthracycline aglycone, η-pyrromycinone[1~3]. The third and fourth band materials were novel and named musettamycin and marcellomycin respectively. Musettamycin crystallized from Skellysolve B - chloroform as dark red plates, m. p. 162~163°C. It and marcellomycin both crystallized from acetonitrile as red-orange needles. In all cases, small amounts of pigment impurities persisted.

Pure musettamycin and marcellomycin were prepared by high-performance liquid chromatography in successive systems on EDTA washed (pH 6.8 buffer) fractions from the Sephadex LH-20 columns. Preliminary purification was carried out in a four column bank of μ-Styragel (Waters Associates, Inc., Milford, Mass.), using chloroform as a mobile phase. Pure musettamycin was prepared by subsequent chromatography on phenyl/Porasil B (37~75 μ) using 35:65 acetonitrile - 0.01 M CH_3COONa (pH 4.0) as the mobile phase with periodic analysis of fractions on a phenyl/Porasil column where the mobile phase was 45:55 acetonitrile - 0.01 M CH_3COONa (pH 4.0). The same process was

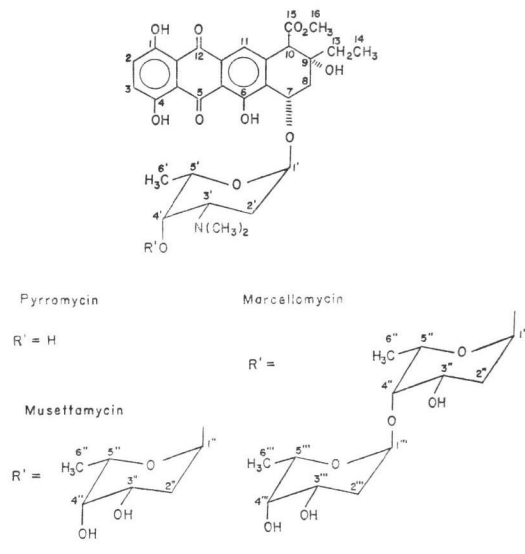


Fig. 1. Ultraviolet and visible absorption spectra of musettamycin and marcellomycin (*c* 0.013, methanol)

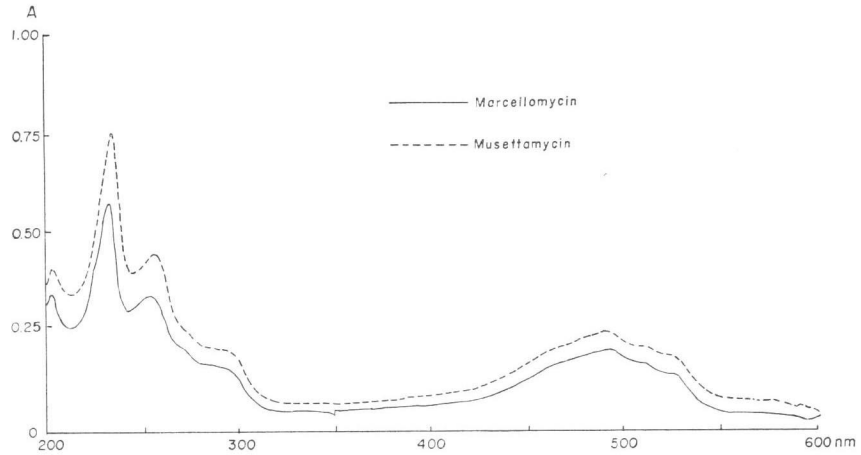
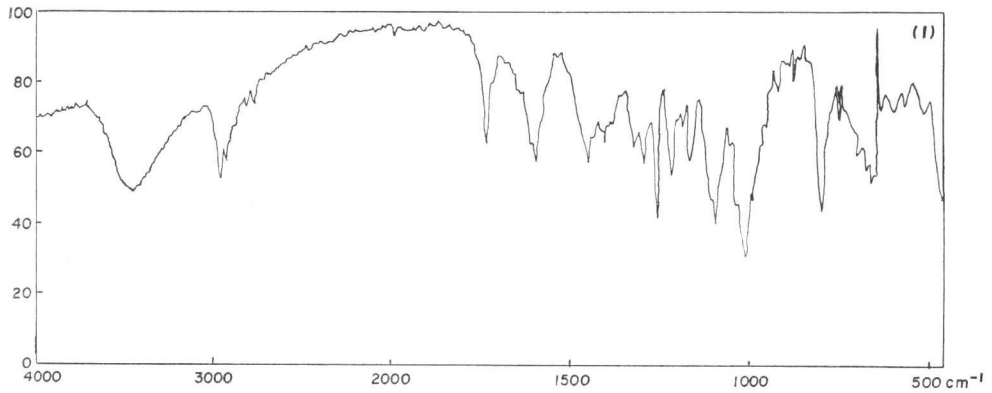
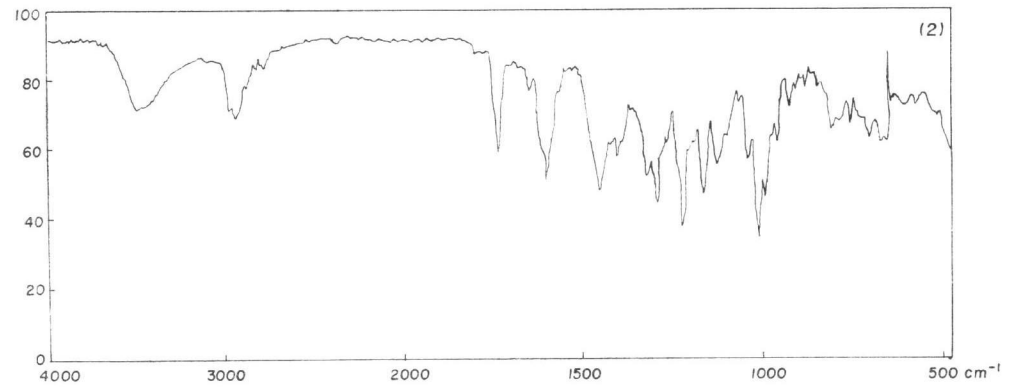


Fig. 2. Infrared absorption spectra of musettamycin (**1**) and marcellomycin (**2**) (KBr pellet)

(**1**)



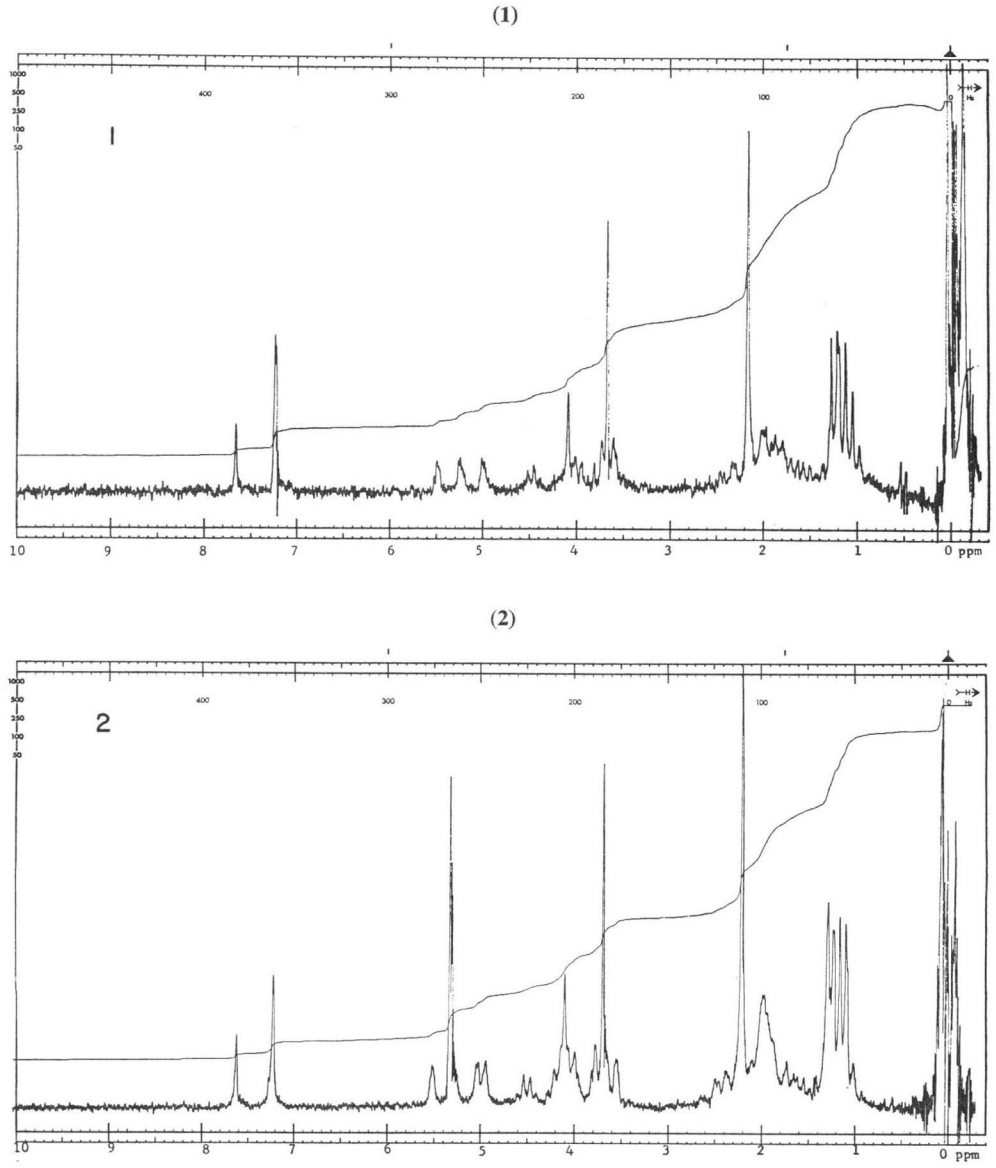
(**2**)



used to obtain pure marcellomycin except that 45: 55 acetonitrile - 0.01 M CH_3COOH (pH 4.0) was the mobile phase for the phenyl/Porasil column.

Upon total acid hydrolysis of both musettamycin and marcellomycin, the known aglycone, ε-pyrromycinone, was generated. Partial cleavage to pyrromycin[4,5] was achieved by alcoholysis in 0.1 N HCl in anhydrous methanol at ambient temperature for 20 hours. Distribution of the evaporated product between D_2O brought to pH 10 with K_2CO_3 and $CDCl_3$ allowed identification of the product by comparison of its 100 mH_ZNMR spectrum with that published[6]. Analysis of the D_2O phase by 100 mH_Z NMR spectrum confirmed the presence of a single sugar which by its spectral characteristics was identified as the methyl glycoside of 2-deoxyfucose. From the NMR spectra of the intact antibiotics it is evident that musettamycin is a disaccharide whereas marcellomycin is a trisaccharide, these having the structures shown before.

Fig. 3. Proton NMR spectra of musettamycin (**1**) and marcellomycin (**2**) (100 mHz, $CDCl_3$)



Physicochemical properties of musettamycin and marcellomycin are as follows:

Musettamycin Anal. calcd. for $C_{36}H_{45}NO_{14}$: C 60.41, H 6.34, N 1.95; found: C 60.27, H 6.50, N 1.99. The ultraviolet and visible, infrared (KBr pellet), 100 mH_z proton magnetic resonance, and FT C^{13} magnetic resonance spectra are given in Figs. 1~4 respectively.

Marcellomycin Anal. calcd. for $C_{42}H_{55}NO_{17}$: C 59.64, H 6.55, N 1.65; found: C 58.77, H 6.77, N 1.82. The ultraviolet and visible, IR (KBr pellet), 100 mH_z proton magnetic resonance, and FT C^{13} magnetic resonance spectra are given in Figs. 1~4 respectively.

Biological data for musettamycin and marcellomycin will be reported elsewhere.

Acknowledgments

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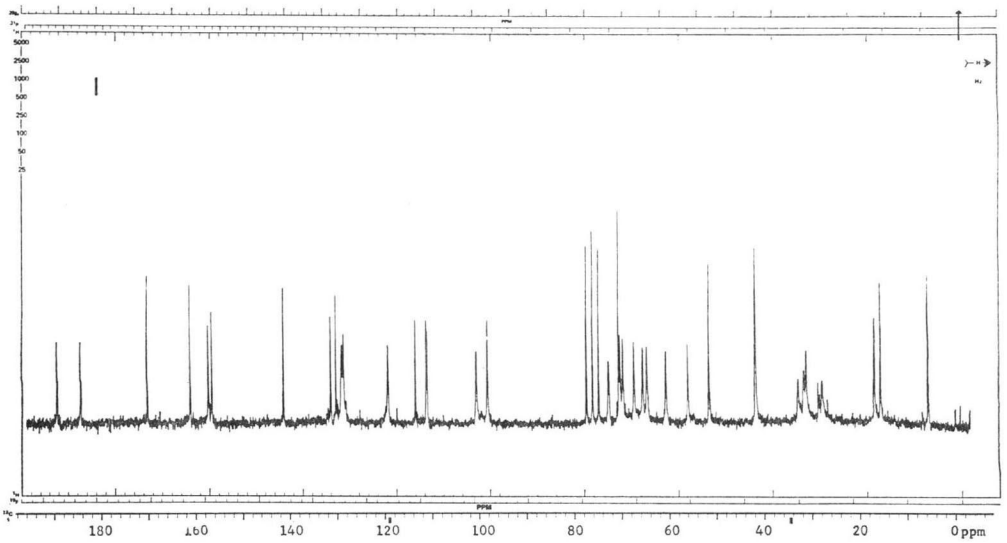
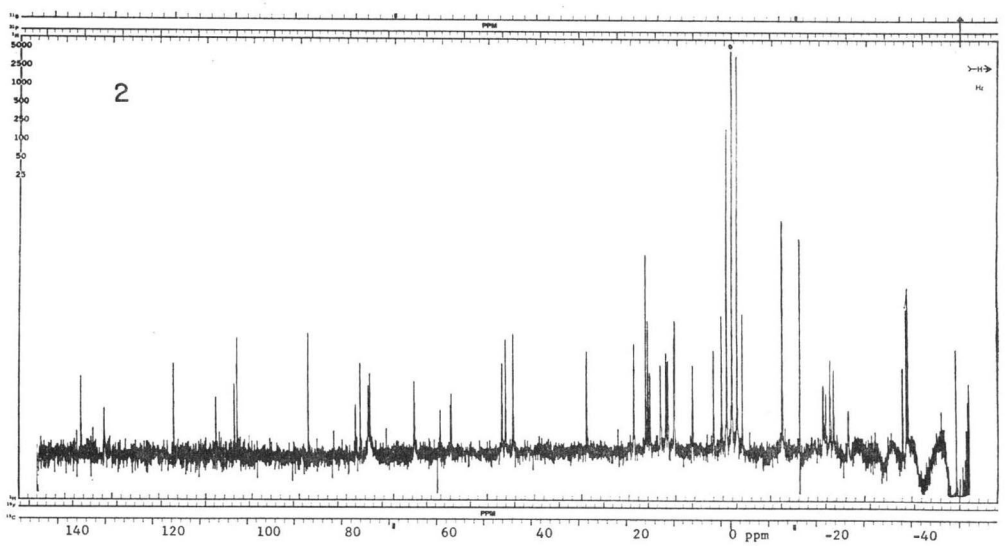
Fig. 4. FT C^{13} magnetic resonance spectra of musettamycin (**1**) and marcellomycin (**2**) (100 mHz $CDCl_3$)

(**1**)



(**2**)



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