AN ADDITIONAL SOURCE OF MACROTETROLIDE ANTIBIOTICS

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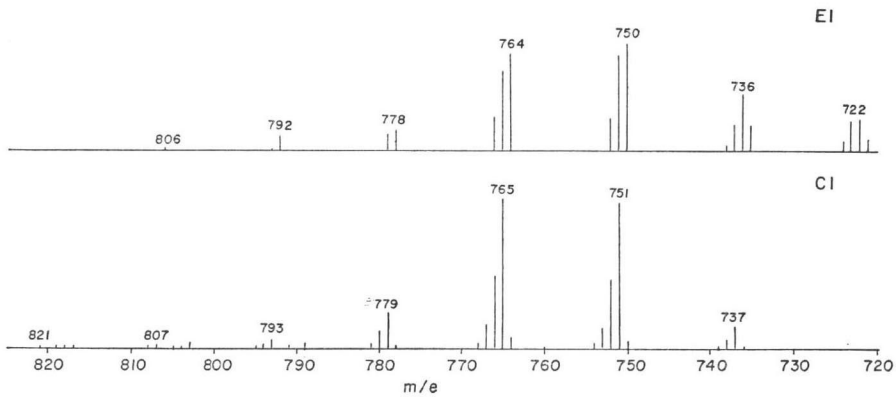
The macrotetrolide antibiotics nonactin, monactin, dinactin, trinactin, tetranactin, and their higher homologs macrotetrolides B,C,D, and G are found in different admixtures and proportions in a variety of *Streptomyces* species.[1] Nonactin has been isolated from *Streptomyces* ETH 7796 (like *S. viridochromogenes* or *S. olivochromogenes*),[2] *S. longispori*,[3] *Streptomyces* No. 10400,[4] *S. chrysomallus*,[5] *S. werraensis*,[6] and *S. tsusimaensis*,[7] whereas nonactin, monactin, dinactin, and trinactin mixtures have been recovered from *Streptomyces* strains ETH A7796, A9828, and A23112[8,9] and from *Streptomyces* strain SC 3763.[10] These four macrotetrolides together with four higher homologs were formed by *S. flaveolus* ETH 31442,[11] and dinactin, trinactin, and tetranactin were found in cultures of *S. aureus* S-3466.[12,13]

Some of the *Streptomyces* species which produce macrotetrolides have been reported to hydroxylate C_{21}-steroids of the pregnane class in the 16α-position, *S. aureus* ATCC 3309, WAKSMAN Collection No. 3569 and No. 3676, and CZAS, *S. chrysomallus* CZAS, and *S. flaveolus* CZAS being instances.[14,15] Furthermore, the antibiotics roseomycin, roseothricin, and seligocidin totally unlike the macrotetrolides have been found in cultures *Streptomyces* strains which resemble *S. roseochromogenus*,[16] a well known species which 16α-hydroxylates steroids.[14,15] However, it is uncertain whether a given *Streptomyces* has the capacity for antibiotic production and C_{21}-steroid 16α-hydroxylation in the same vegetative cell culture. The production of a complex mixture of macrotetrolides concommitantly with active 16α-hydroxylation of steroids by a strain of *S. roseochromogenus* (*roseochromogenes*) WAKSMAN Collection No. 3689[17~20] is reported herein.

The macrotetrolide preparation obtained as a crystalline mass from mycelium in the usual fashion following 16α-hydroxylation of 9α-fluoro-11β, 17α, 21-trihydroxypregn-4-ene-3, 20-dione[17,20] inhibited Gram-positive bacteria such as *Staphylococcus albus* and *aureus* and *Streptococcus faecalis* and *pyogenes* at 1~2 μg/ml concentration but was inactive against a variety of Gram-negative organisms. This differential sensitivity of Gram-positive bacteria towards the macrotetrolides characterizes this class of antibiotics.[1] The preparation was recognized as probably being dinactin from its several physical properties and from elemental analyses, but the composition of the sample was clearly demonstrated complex by thin-layer chromotography. Despite the technical problems of application of thin-layer

Fig. 1. Molecular ion region of EI and CI mass spectra of the macroterolides of *S. roseochromogenus*.



chromatography to these antibiotics[1,21] it was possible to demonstrate the presence of dinactin and monactin as major components, with smaller amounts of nonactin and trinactin suggested also.

A complete analysis of the macrotetrolide sample was achieved *via* mass spectrometry, a means well recognized as necessary for ultimate analyses of this class of antibiotics.[1,11,21] Mass spectra of the macrotetrolide sample obtained *via* electron impact (EI) and chemical ionization (CI) modes presented in Fig. 1 for the high mass region of the molecular ions clearly establish the complexity of the sample and of the predominance of dinactin and monactin as major components.

The macrotetrolide antibiotics are characterized by their mass spectra wherein the molecular ions M^+ and the fragmentation ions *m/e* 553, 567, 581, 595, 609, and 623 in the 3/4 M+1 region (corresponding to fragments M−183, M−197, and M−211), the ions *m/e* 369, 383, 397, 411, and 425 in the 1/2 M+1 region, and the ions *m/e* 185, 199, and 213 in the 1/4 M+1 region are prominent.[1,11,21] Inspection of the EI mass spectrum of Fig. 1 clearly discloses clusters of ions at *m/e* 792, 778, 764, 750, and 736 associated with molecular ions of tetranactin and/or macrotetrolide D, trinactin and/or macrotetrolide G, dinactin, monactin, and nonactin respectively. A very weak ion at *m/e* 806 suggests the presence of traces of macrotetrolide C as well.

The suggested identities are confirmed by inspection of the 3/4 M+1, 1/2 M+1, and 1/4 M+1 regions. Prominent fragmentation ions at *m/e* 581, 567, and 553 infer the prodominance of dinactin, monactin, and nonactin in the sample, and much weaker ions at *m/e* 623, 609, and 595 suggest much lower levels of the higher homologs trinactin, tetranactin, and the macrotetrolides C, D, and G from which these ions derive. Furthermore, the 1/2 M+1 region of the EI mass spectrum includes prominently the ions 383 and 369 associated with the lower homologs and the less intense ions *m/e* 425, 411, and 397 associated with the higher homologs. Very intense ions at *m/e* 199 and 185 but a modest *m/e* 213 ion fully confirm the composition of the sample as being enriched in the lower homologs dinactin, monactin, and nonactin.

These identities are confirmed by the CI mass spectrum where the quasimolecular ions $(M+1)^+$ are prominent rather than the molecular ions, which are weak though present. The relative composition of the macrotetrolide sample was estimated from replicate measurements of the relative abundances of the several molecular $(M)^+$ and quasi-molecular $(M+1)^+$ ions in the EI and CI mass spectra respectively, as follows: nonactin, 22%, 6%; monactin, 33%, 40%; dinactin, 33%, 41%; trinactin and/or macrotetrolide G, 9%, 9%; tetranactin and/or macrotetrolide D, 3%, 2%; macrotetrolide C, <1%, <1%. Thus, with the exception of the estimates of amount of nonactin in the sample for which agreement was not good, the estimated composition of the sample determined by EI was in agreement with the composition determined by CI.

In addition to these components traces of macrotetrolide B are evinced by the ion *m/e* 821 of the CI spectrum. The ion cluster at *m/e* 722 in the EI spectrum but missing from the CI spectrum probably represents EI fragmentations not occurring by CI.

Experimental Section

Thin-layer chromatography was conducted on 0.25 mm thick 20×20 chromatoplates of Silica Gel HF_{254} (E. Merck. GmbH., Darmstadt) using chloroform-ethyl acetate (1:1 and 1:2) and iodine vapors and 50% sulfuric acid spray for detection. Mass spectra were recorded with a Du Pont Model 21-491 mass spectrometer equipped with a solid sample inlet probe, using electron energy of 70 eV for both EI and CI. Isobutane was used as reagent gas for CI mass spectra.

A 24-hour vegetative cell culture of a strain of *S. roseochromogenus* WAKSMAN Collection No. 3689 grown in soybean meal, soybean oil, glucose medium containing calcium carbonate and adjusted to pH 7.0[17,18,16] to which 9α-fluoro-11β, 17α, 21-trihydroxypregn-4-ene-3, 20-dione (260 μg/ml) was added was aerated at 25°C for 72 hours. Mycelium from 29 liters of broth was recovered by filtration with diatomaceous earth, the mycelial cake washed with water, and the moist cake extracted with 50 liters of refluxing methanol for 2 hours. The hot methanol extract was filtered and evaporated under vacuum to 2.5 liters, filtered

from insoluble material, and cooled overnight. The sludge which precipitated was filtered, dissolved in pentane, and the solution was refiltered. The pentane filtrate was allowed to evaporate slowly at room temperature for a week, during which time the macrotetrolides crystallized. The crystalline product was decanted, triturated with pentane to dissolve oily material, and filtered, yielding 1.8 g of crystalline macrotetrolides, m. p. 72~76°C (KOFLER block); $[\alpha]_D$+0.8° (EtOH); no selective absorption above 195 nm; ν_{max}^{KBr} 2941, 1736, 1460, 1379, 1266, 1198, 1117, 1060, 812 cm^{-1} very similar to published spectra of the macrotetrolides;[2,4,5,6,7,13,21] NMR (in $CDCl_3$) δ 1.11 (*d*, *J*=6 cps), 1.27 (*d*, *J*=6 cps), 1.76 (*t*, *J*=6 cps), 4.07 (broad), 5.05 (m).

Anal. Calcd. for $C_{42}H_{68}O_{12}$: C, 65.94; H, 8.96; O, 25.10; M, 764. Found: C, 65.63; 9.01; O, 25.30; M, 794 (chloroform evaporation method). Reprocessing of the pentane filtrates and washes two more times yielded an additional 6.9 g of macrotetrolides. The original aqueous broth filtrate and water washes of the mycelium were combined and extracted with methyl isobutyl ketone,[17] the extract evaporated, and steroids thereby recovered in the usual fashion. 9α-Fluoro-11β, 16α, 17α, 21-tetrahydroxypregn-4-ene-3, 20-dione and its D-homoannulated rearrangement product were identified by chromatographic and spectral properties.[22,23]

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