# THE IDENTITY OF THE ANTIBIOTICS NIGERICIN, POLYETHERIN A AND X-464

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The isolation of three crystalline antibiotics from three different streptomyces species was reported from this laboratory[1] in 1951. These compounds, referred to as X-206, X-464 and X-537A, exhibited similar biological activity but most unusual was the fact that their alkali metal salts were soluble in such non-polar solvents as benzene and petroleum ether and virtually insoluble in water. At the same time, the isolation of nigericin[2], a compound with similar biological and chemical properties, was disclosed. Recently, we have been re-examining these compounds in an attempt to elucidate their structures. While this work was in progress, two simultaneous publications[3,4] based on X-ray crystallography indicated identical structural formulas for nigericin and polyetherin A. On the basis of the published data, the identity of these two compounds was not firmly established since there could be differences in the stereochemistry. LARDY[5] had also reported that GORMAN had shown that nigericin and X-464 were identical; however, in a subsequent publication, GORMAN[6] indicated only that the two samples could not be separated by paper chromatography. SHOJI *et al.*[7] compared polyetherin A with nigericin and X-464 and found them to be different. We now wish to present conclusive evidence that nigericin and polyetherin A are the same and are also identical with antibiotic X-464. Since the earliest name assigned in the literature was nigericin, it is proposed that this name be retained in future publications.

Thin-layer chromatography of the original sample of X-464[1] showed the presence of a more polar impurity. This biologically active impurity was also observed in samples of the sodium salt of nigericin but not in a polyetherin sample. Recrystallization of X-464 from a mixture of methylene chloride and hexane gave one spot material of $[\alpha]_D^{25}$ $+35.2°$ ($c$, 0.9, chloroform) and $[\alpha]_D^{25}$ $+9.21°$ ($c$, 1.03, methanol) both in good agreement with the values reported for polyetherin A[7] and identical within experimental error with values obtained on a sample of nigericin (free acid). Similarly, the sodium salts of all three samples had $[\alpha]_D^{25}$ $+7.8°$ ($c$, 1.05, methanol).

The infrared spectra of the sodium salts and the free acids of the three samples in chloroform solution were identical and in good agreement with the published spectra of polyetherin A.

Determination of the optical rotatory dispersion of X-464 sodium salt in methanol showed a positive COTTON effect with a peak at $[\phi]_{214}+3660°$ and a trough at $[\phi]_{203}$ $+3120°$. The free acid showed no COTTON effect at wavelengths higher than 190 m$\mu$. Samples of all three antibiotics, as the sodium salts, exhibited the same COTTON effect, within the limits of the experimental method. Therefore, nigericin and polyetherin A both have the same molecular structure, from X-ray crystallography, and the same configuration, from ORD measurements.

A molecular weight determination by osmometry in chloroform on X-464 (free acid) gave a value of $711\pm14$, in good agreement with the calculated value of 724. The free acid was unsuitable for molecular weight determination by mass spectrometry because of extensive thermal decomposition under the experimental conditions. However, both the $Na^+$ and $K^+$ salts gave molecular ions (m/e=746 and 763 respectively) although these salts showed some decomposition. Similar fragmentation patterns were obtained with the corresponding salts prepared from samples of nigericin and polyetherin A. The small differences in the spectra can be caused by concomitant decomposition. Mass spectra of the $Na^+$ and $K^+$ salts of the three antibiotic samples also showed a minor peak at $(M+23)^+$ and $(M+39)^+$ respectively suggesting the presence of ions in which the antibiotic is associated with two metal atoms.

These ions may be formed in the mass spectrometer and may have no corresponding species in the solid state. Further, the $Na^+$ salt of a monoacetyl derivative of X-464* also gave a molecular ion, (m/e=788) and showed less thermal decomposition in the mass spectrometer than the antibiotic salts, probably due to greater volatility. The highest peak observed in the mass spectrum of the methyl ester of X-464 and nigericin was at m/e=720, corresponding to $(M-H_2O)^+$. This is contrary to the observation that polyetherin A methyl ester gives a molecular ion on electron impact[4].

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The identity of nigericin and polyetherin A was also reported by S. MATSUTANI and T. KUBOTA at the 22nd Meeting of the Japan Chemical Society held on April 5, 1969 in Tokyo (Abstract paper, Part III, page 1897). (Editor)

* Crystallized from hexane as colorless plates, m. p. 240～250° dec. Anal. Calcd. for $C_{42}H_{69}O_{12}Na$ (788.9) : C 63.94, H 8.81, Na 2.91; Found : C 63.65, H 8.72, Na 2.88; $[\alpha]_D^{25}$ +3.2 (*c* 0.9, methanol), $[\alpha]_D^{25}$ +8.6 (*c* 1.0, chloroform).