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DETECTION OF ISODINACTIN BY NMR SPECTROSCOPY

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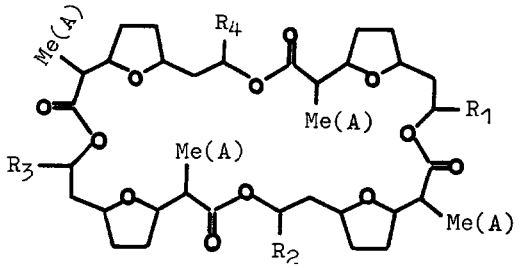
Macrotetrolide antibiotics, nonactins[2,3] (Fig. 1), are well known to exhibit the cation selectivity[4] in the complex formation with alkali metal ions and in the ion transport through biological membranes. Molecular structures of nonactin[5] and tetranactin[6] have been determined by X-ray crystal analyses to reveal that the two homologues are of the same structures of Form[I] (Fig. 2). Structure analyses for crystals of K^+ complexes[7] of nonactin and tetranactin by the X-ray diffraction method have indicated that the complexes adopt similar conformations, that the outline of the 32 membered ring of the complexes resembles the seam of a tennis ball, and that central ions are surrounded by the eight oxygen atoms to form a distorted cubic coordination.

Mass spectra of dinactin[8] have indicated, for the first time, that the sample was accompanied by its isomer of the Form[II] (Fig. 2), of which fragmentation patterns at the $M^+/2$ region (relative intensities at m/e of 369, 383 and 397) are expected to be 1:2:1, and for dinactin molecule of the Form[I] 0:4:0.



tetranactin : $R_{1,2,3\ \&\ 4} = -C_2H_5$.
trinactin : $R_{1,2\ \&\ 3} = -C_2H_5$, $R_4 = -CH_3$.
isotrinactin : $R_{1,2\ \&\ 4} = -C_2H_5$. $R_3 = -CH_3$.
dinactin : $R_{1\ \&\ 3} = -C_2H_5$, $R_{2\ \&\ 4} = -CH_3$.
isodinactin : $R_{1\ \&\ 2} = -C_2H_5$, $R_{3\ \&\ 4} = -CH_3$.
nonactin : $R_{1,2,3\ \&\ 4} = -CH_3$.

Fig. 1. Structures of macrotetrolide antibiotics.

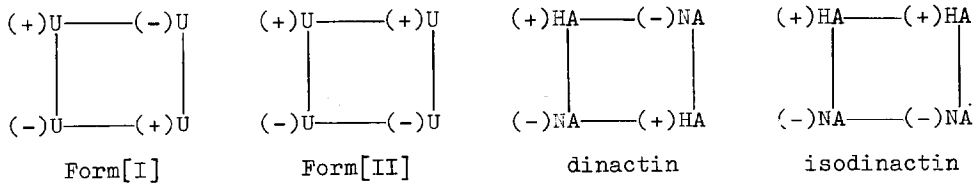


Fig. 2. Schematic constitutions of macrotetrolide antibiotics.
subunit (U) : homononactic acid(HA) ($R_i = -C_2H_5$, in Fig. 1).
nonactic acid(NA) ($R_i = -CH_3$, in Fig. 1).
optical rotation : levo = (-), dextro = (+).

In NMR spectra of nonactin, which is very flexible in solution, averaged signals have been observed as the result of rapid molecular motion.[9] However, it may be expected that the intramolecular motions of the complexes are restricted to minor structural changes, and that the conformations of the complexes of the Forms[I] and [II]can be preserved stably in solution.

In the present letter, proton signals characteristic of the isomers in NMR spectra of K^+ complexes of the antibiotics are reported.

The samples of tetranactins used in this experiment were fractionated by the column chromatography.[10] Authentic samples of nonactin, dinactin, and trinactin were generously presented by Prof. W. Keller-Schierlein of E.T.H. in Zürich. K^+ complexes of the antibiotics with KSCN were prepared in CD_3OD solutions containing a slight excess amount of the salt. Mass spectra of the antibiotics were measured by a double-focus-type Hitachi RMU-6E, and NMR spectra were recorded on a Hitachi Perkin-Elmer R-20A spectrometer (60 MHz, in CD_3OD, TMS internal standard).

In the mass spectra of dinactin samples of E.T.H. and of this laboratory (Chugai), the relative intensities at the $M^+/2$ region were indicated in ratios of 1.9:7.2:1.0 and 2.4:14.5:1.0, and approximate compositions (dinactin:isodinactin) were 1:1 and 3:1, respectively. But, as it was expected, no detectable difference was observed in the mass spectra of trinactin samples of E.T.H. and Chugai.

In NMR spectra of dinactin in CD_3OD [Fig. 3(c) and (d)], doublet proton signals due to methyl groups adjacent to a carbonyl group in the subunits (Me(A), in Fig. 1) became broader and the peak heights were extraordinarily diminished in comparison with those in a $CC\ell_4$ solution,[2] since the differences in intramolecular environments around Me(A) protons of dinactin and isodinactin may be enhanced in the polar solvent.

A downfield shift ($\Delta\delta$= 0.09 ppm) of Me(A) signals of tetranactin was observed in the course of complex formation [Fig. 3(a)]; doublet signals of Me(A) in K^+-tetranactin, T(d), appeared at δ 1.17 (J= 7.0 Hz), and those of K^+-nonactin, N(d), were found at δ 1.13 (J= 6.9 Hz) [Fig. 3(b)]. In the crystal structures of the complexes, a slight difference was observed in the averaged distances between K^+ and oxygen atoms of tetrahydrofuran rings,

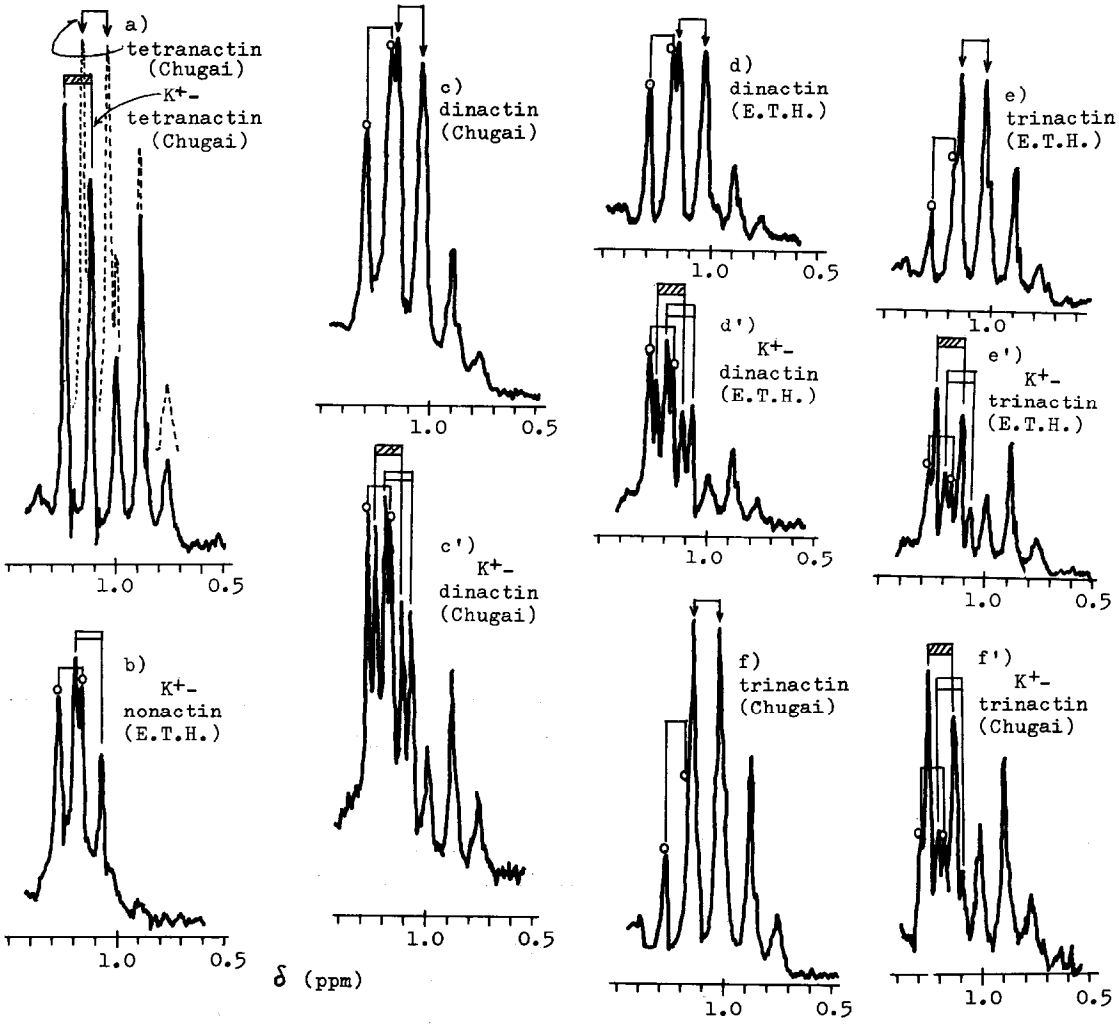


Fig. 3. Methyl proton signals in NMR spectra of macrotetrolide antibiotics in CD_3OD (TMS internal standard). T(d) and N(d) types of doublet methyl signals are indicated by and , respectively. =Me(A) signal, and =methyl (R_i, in Fig. 1) signal.

where R_i (in Fig. 1) may make the Me(A) protons magnetically nonequivalent between the two homologues.

Both T(d) and N(d) types of Me(A) signals were observed in the spectra of K^+-dinactin complexes [Fig. 3(c') and (d')]; but the relative peak heights of T(d) to N(d) type signals in the sample of dinactin of E.T.H. were lower than those of dinactin of Chugai, which is rich in dinactin. This fact suggests that the Me(A) protons in isodinactin show the signals of N(d) type, whereas those in dinactin exhibit the signals of both types of T(d) and N(d). Accordingly, a pair of Me(A) groups attached to the subunits of dinactins which were related with an approximate twofold axis in the molecule, (-)NA····K^+····(-)NA or (-)NA····K^+····(+)HA, may give N(d) type signals, whereas those in the structure of (+)HA····K^+····(+)HA may give T(d) type signals. These results are the second experimental evidence for the coexistence of isodinactin in dinactin samples.

Although analogous spectra were observed for trinactin samples, it was uncertain whether the spectra of the K^+ complex indicate the existence of isomer of trinactin (especially, N(d) type signals) [Fig. 3(e), (e'), (f), and (f')].

The isolation of the isomers of dinactin and trinactin as K^+ complexes is now in progress by the column chromatography. The results will be reported elsewhere.

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