TETRANACTIN, A NEW MITICIDAL ANTIBIOTIC
II. STRUCTURE OF TETRANACTIN

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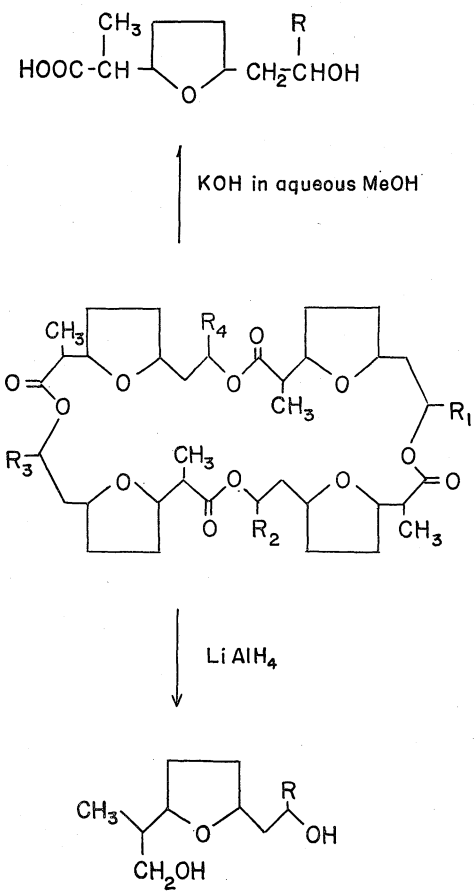
Tetranactin, m.p. 105~106°C, $[\alpha]_D^{23.5}$ 0 (*c* 1, chloroform), belongs to the macrotetrolide antibiotic class, as exemplified by nonactin. The molecular formula, $C_{44}H_{72}O_{12}$, was assigned on the basis of elementary analysis and the molecular weight determined by mass spectroscopy. Tetranactin consists of four units of homononactic acid linked to form a cyclic polyester. Crystal data from X-ray photograph indicate that the tetranactin molecule is either a racemic or meso-form.

Isolation, characterization and some properties of tetranactin have been reported previously[1,2]. Tetranactin is the first miticidal antibiotic with potential practical usefulness in view of the remarkable activity and low toxicity to a warm-blooded animal. This paper deals with the structure of tetranactin.

Fig. 1. Structures of macrotetrolide antibiotics and the constituents.

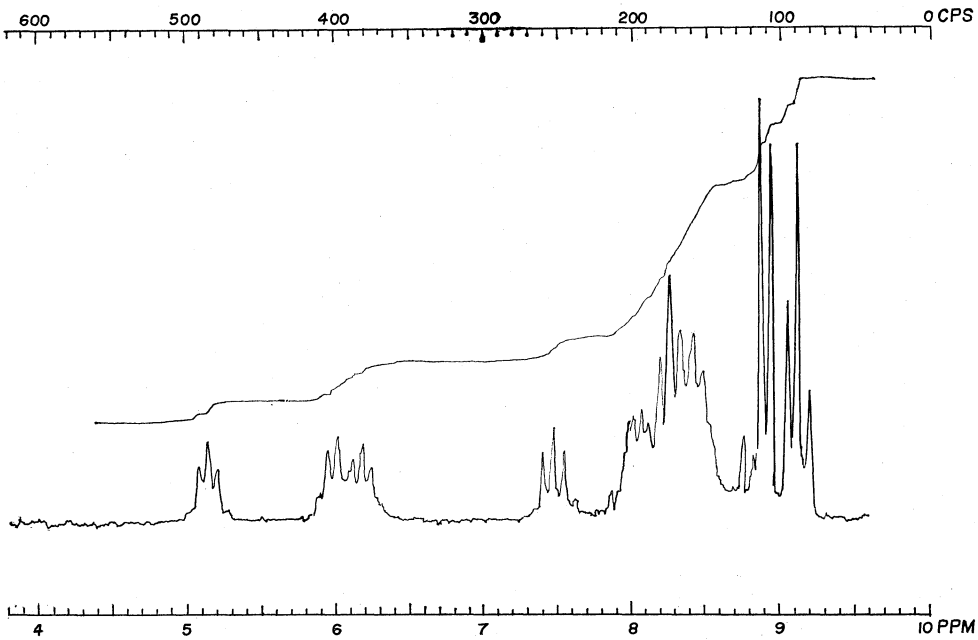


Nonactic acid (I)
R=methyl
Homononactic acid (II)
R=ethyl
Bishomononactic acid (III)
R=isopropyl

Tetranactin (IV)
$R_{1,2,3 \text{ and } 4}$=ethyl
Nonactin (V)
$R_{1,2,3 \text{ and } 4}$=methyl

Streptomyces aureus strain S-3466, isolated from a soil, produced an insecticidal antibiotic in the fermented broth when Azuki-bean weevil, *Callosobruchus chinensis*, was used as a test insect in the screening. The active principle, tetranactin, was isolated in crystalline form by extracting the filter cake of the fermented broth with acetone followed by silica-gel column chromatography. The antibiotic was shown to be closely related to the macrotetrolide antibiotics, as exemplified by nonactin[3]. Nonactin was so named because of the lack of

Fig. 2. NMR spectrum of tetranactin (in $CDCl_3$)

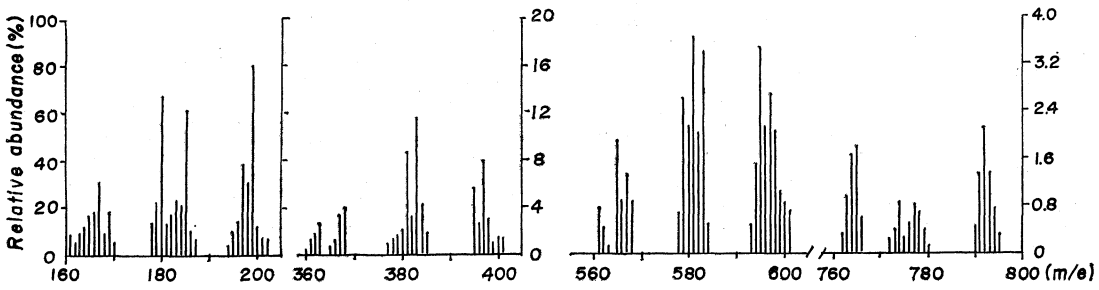


biological activity. The molecule of nonactin contains four units of the hydroxycarboxylic acid, nonactic acid (I), linked to form cyclic polyester (Fig. 1).

The crude tetranactin showed three spots on silica-gel thin-layer chromatography even after repeated recrystallization. Pure tetranactin was separated by silica gel column chromatography from the other two components, A and B, which are also structurally related to the macrotetrolide antibiotics. The infrared absorption spectrum of tetranactin showed strong bands characteristic of the macrotetrolides.

The nuclear magnetic resonance spectrum of tetranactin is shown in Fig. 2. The spectrum of tetranactin is very similar to that of nonactin[4], but marked differences were observed in the signals at τ 8.7 and 9.1. A doublet at τ 8.7 in nonactin is not found in the spectrum of tetranactin, but is replaced by a triplet at τ 9.1 which is absent in the spectrum of nonactin. Three kinds of constituent hydroxycarboxylic acids are known for macrotetrolide antibiotics; that is, nonactic (I), homononactic (II) and bishomononactic (III) acids. The doublet at τ 8.7 is due to the methyl groups of

Fig. 3. Mass spectrum of tetranactin.



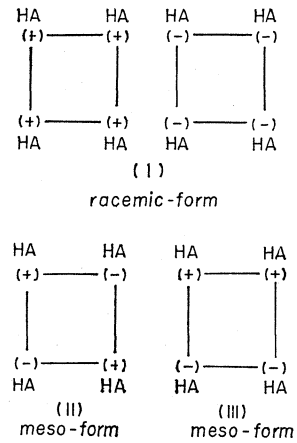
nonactic acid moieties attached to a carbon bearing ester-bonded oxygen (R-CH-O-COR). The
CH_3
triplet at τ 9.1 (12 H, 4 methyl in tetranactin) corresponds to the methyl groups of homononactic acid moieties attached to methylene (R-CH-O-COR′), suggesting that tetranactin
CH_2-CH_3
contains four units of homononactic acid. The doublet at τ 8.9 is the signal of methyl attached to methine adjacent to ester carbonyl (R-O-CO-CH-). The triplet at τ 5.15 is due to
CH_3
the methine bearing ester bonded oxygen (R-CH-O-CO-R′), the shape and coupling constant (J=6 cps) of wnich are slightly different from those of nonactin (multiplet).

Table 1. Crystal data of tetranactin

Solvent for crystallization	Ethylacetate
a [Å]	25.40
b [Å]	9.48
c [Å]	24.49
α°	90.0
β°	129.69
γ°	90.0
Cell volume [Å^3]	4538
Z*	4
ρ cal	1.16
ρ obs**	1.166
Systemic absence	for (kkl) $k+k\neq 2n$ for (kol) $1\neq 2n$
Space group	C 2/c

* Z=numbers of molecules per unit cell.
** Densitiy measurement was carried out by floatation method in the aqueous solution of KI.

Fig. 4. Stereochemical models of tetranactin.
HA : homononactic acid unit.



The mass spectrum of tetranactin is shown in Fig. 3. KELLER-SCHIERLEIN and co-workers[5] reported that the parent peaks appeared in the mass spectrum of the macrotetrolide in spite of the relatively high molecular weight and the high proportion of oxygen content, when the samples were heated over 180°C using direct inlet system. Therefore, mass spectroscopy is the most useful tool for identification of macrotetrolide antibiotics now available, because the molecular weight can be conclusively determined and the fragmentation patterns indicate the two-dimentional structure of the antibiotic. As expected, the molecular ion peak appeared at *m/e* 792 in the spectrum of tetranactin. The characteristic rearrangement peaks for the macrotetrolide were present at *m/e* 199, 383, 397 and 595. These peaks are due to homononactic acid moieties fragmented from the ring by double fission.

The molecular formula, $C_{44}H_{72}O_{12}$, was assigned for tetranactin on the basis of the molecular weight and the elementary analysis. Tetranactin has the same molecular formula as substance D found by KELLER-SCHIERLEIN[5]. Substance D consists of one nonactic acid, two homononactic acid and one bishomononactic acid moieties. If tetranactin were identical with substance D, the nmr spectrum should show a doublet at τ 8.7 (3H), and an overlaped signal of triplet (6H) and doublet (6H) at τ 9.1. However, only a triplet signal at τ 9.1 (12H, 4 methyl) is present in the spectrum. Thus, tetranactin is a new antibiotic composed of four homononactic acid moieties, as shown in Fig. 1.

Validity of this assumption was confirmed by $LiAlH_4$ reduction and alkaline hydrolysis. Upon $LiAlH_4$ reduction tetranactin yielded the diol (**VI**) as the sole reaction product, the structure of which was determined by IR, nmr, mass spectra and elementary analysis. Alkaline hydrolysis of tetranactin yielded homononactic acid and small amounts of the epimer, thus supporting the structure presented.

Determination of three dimentional structure is important in macrotetrolide antibiotics, since 2^{16} stereoisomers are possible for the structure. Crystal data from X-ray photographs, taken by Weissenberg and precession cameras with CuK_α radiation on tetranactin, is shown in Table 1. Tetranactin is readily crystallized from ethylacetate forming large prisms of the monoclinic system. From the extinction of reflections, the space group of the crystal was either Cc or C2/c. While the N(2) test on the (*kko*) reflections (in all 86, excluding (*koo*) and (*oko*)) indicated the existence of center of symmetry (Fig. 4), so the space group was assigned to C2/c. Accordingly, tetranactin molecule has either a two-fold rotational axis (racemic molecule **I** or meso form **II**) or center of symmetry (meso form **III**). This is consistent with the data of the optical rotation, $[\alpha]_D^{23.5}$ 0 (*c* 1, in chloroform). Crystal structural analysis of tetranactin as well as the other two components is now in progress by applying the symbolic addition method and the result will be presented elsewhere.

The molar ratio of tetranactin : component A : component B in the fermented broth varied according to the fermentation conditions. However, nonactin could not be detected in any fermented broth with the present organism, *Streptomyces aureus* S-3466. This is the first observation that homononactic acid is the major constituent in macrotetrolide antibiotic production by fermentation. The detailed miticidal activity of tetranactin will be presented in subsequent papers[6,7].

Experimental

Measurement of the spectra : Mass spectra were taken with Hitachi RMU-6E mass spectrometer. Japan Electron Optics JNM-3H-100 nuclear magnetic resonance spectrometer was used for measurement of nmr spectra in $CDCl_3$ using tetramethyl silane as an internal standard. IR spectra were recorded by Japan Spectroscopic Co. Model IR-S. Ultraviolet absorption spectra were obtained by Cary Model 11 M.

$LiAlH_4$ Reduction of tetranactin : Tetranactin (50 mg) was dissolved in a small amount of diethyl ether and the reaction mixture was cooled to 5°C in an ice bath. Powdered $LiAlH_4$ (200 mg) was gradually added to the mixture. After addition was completed, the reaction mixture was heated under reflux for 3 hours. Then, the mixture was acidified to pH 2.0 with dil. HCl and twice extracted with diethyl ether. The ether extracts were combined and dehydrated with anhydrous sodium sulfate. Concentration of the extract *in vacuo* yielded diol (**VI**) as an oil (37 mg).

Calcd. for $C_{11}H_{22}O_3$; C 65.31 %, H 10.96 %, found C 65.21 %, H 10.58 %.

The molecular weight determined by mass spectroscopy was 202.

Alkaline hydrolysis of tetranactin : Tetranactin (1,000 mg) was dissolved in 100 ml of 80 % aqueous methanol containing potassium hydroxide (10 g) and the reaction mixture was heated under reflux for 5 hours. Then, dil. HCl was added to pH 2.0 and the mixture was concentrated *in vacuo* to a small volume. The residue was extracted with diethyl ether and the combined extracts were dehydrated with anhydrous sodium sulfate. Concentration of the extract *in vacuo* yielded an oil (870 mg) which consisted mainly of

homononactic acid with small amounts of the epimer. Purified homononactic acid was obtained using Sephadex LH-20 column chromatography.

Calcd. for $C_{11}H_{20}O_4$; C 61.09 %, H 9.32 %, found C 62.05 %, H 9.51 %.

The molecular weight of the methyl ester, $C_{12}H_{22}O_4$, determined by mass spectroscopy was 230.

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