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STUDY ON BIOSYNTHESIS OF AUREOTHIN, A NITRO-CONTAINING METABOLITE FROM STREPTOMYCES LUTEORETICULI USING ^{13}C-NMR SPECTROMETRY

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Streptomyces luteoreticuli Katoh et Arai (strain KS 2-74) isolated in this institute by Arai et al. from the soil collected in Beppu, Japan, is known to produce specifically some nitro-containing metabolites, e.g., aureothin, luteoreticulin etc.[1] On the source of nitro group in aureothin biosynthesis, a good participation of p-nitro and p-aminobenzoic acids has been reported by Kawai et al.[2] Accordingly, it would probably be considered that the polyketide from acetate-malonate or propionate condenses with p-nitro or p-amino (which may subsequently be oxidized to nitro) benzoic acids to form a particular type of compound of the general form, aryl-$(C=C)_n$-pyrone, as illustrated in Fig. 1.

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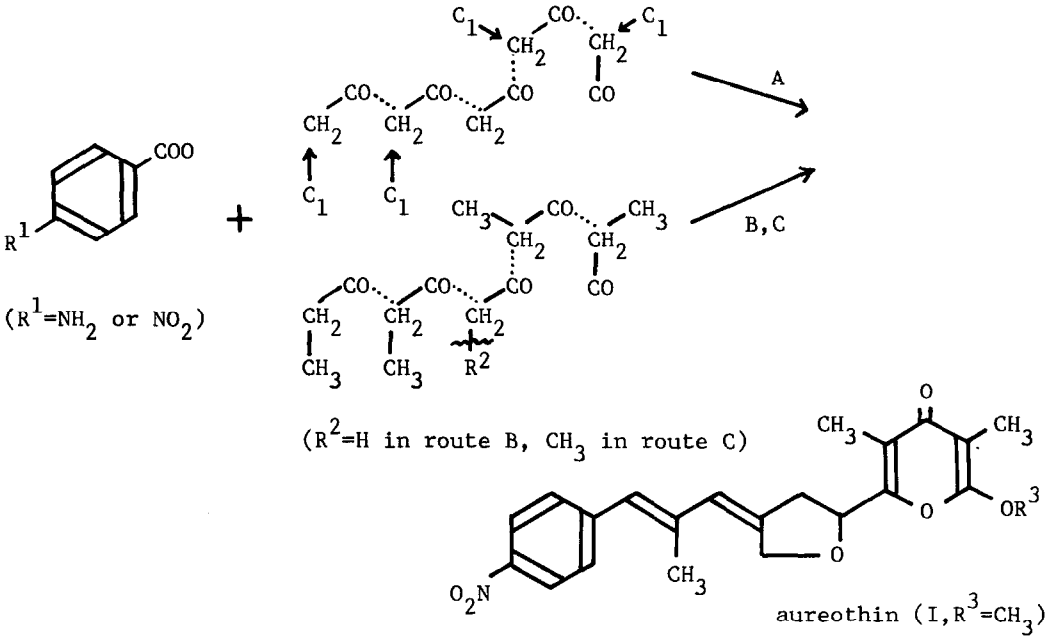
Fig. 1. Possible routes for the aureothin biosynthesis



The first possibility of the biosynthesis through route A might be ruled out because the negative introduction of "extra methyl" from the radioactive methionine has been detected and also a good incorporation of radioactive propionate to aureothin and luteoreticulin has been obtained.

methionine ·····→	aureothin (I, R^3=CH_3)	
1.28×10^{10} dpm/m mole	1.77×10^{8} dpm/m mole	desmethylaureothin (I, R^3=H)
	↓	
	6.31×10^{6} dpm/m mole —HCl-EtOH→	0.0015×10^{6} dpm/m mole
	(diluted with carrier)	

The facts as mentioned above led us to examine whether propionate is able to participate directly in the formation of these metabolites by the use of ^{13}C-NMR spectrometric method.

The yield of aureothin was found reduced by substitution of the acids such as acetate or propionate in place of glucose as the carbon source in the medium.

The reduction of the yield is more significant when acetate is added together with glucose to the medium rather than propionate.[3)]

Sodium propionate-3-^{13}C (500mg, 64% excess, purchased from Sharp and Dohm of Canada) was added to the culture flasks containing 500 ml of glucose-bouillon medium. Aureothin and luteoreticulin were isolated by Al_2O_3 column chromatography of the $CHCl_3$ soluble part of the acetone extract from mycelia after shaking culture[1)] for 48 hours at 27-30°C.

For the measurement of ^{13}C-NMR spectra of aureothin of natural abundance and of enriched by addition of ^{13}C-propionate to the medium, PS-100 high resolution NMR spectrometer equipped with Fourier transform system was used. The ^{13}C-NMR spectra using proton noise decoupling technique obtained are shown in Fig. 2. The assignment of each of the carbon resonance was proposed by the comparison of chemical shifts and the splitting pattern observed in off-resonance continuous wave decoupling measurement. The data and information on the assignment of the carbon resonance will be reported elsewhere.

Obviously from the result shown in Fig. 2, the source of carbons of four "extra methyl"s in aureothin has been confirmed to be derived biosynthetically from the methyl of the propionate administered according to the route B or C as shown in Fig. 1. Since the incorporation of radioactive acetate as well as propionate to aureothin and luteoreticulin was observed, a direct participation of acetate together with propionate might be thought being much probable (route B). In route C, a splitting of one methyl (R^2 in Fig. 1.) after derivation from the methyl of propionate should be accomplished.

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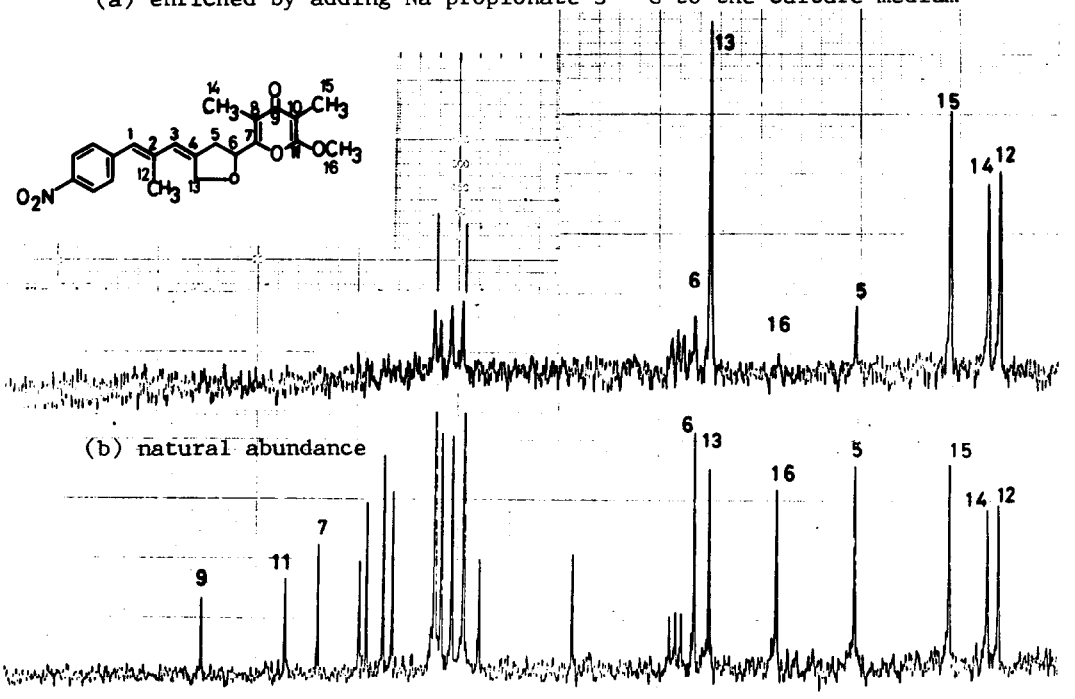
Fig. 2. ^{13}C-FT-NMR spectra of aureothin

JNM-PFT-100 (25.15 MHz) with EC60 computer



F=6112.5 Hz Sample: (a) 12.3 mg/0.5 ml, (b) 400 mg/1.0 ml$CDCl_3$

tw=11 μsec. (45°) Repetation=4 sec x 200 times

Ref.=$CDCl_3$ from CS_2 Lock=$CDCl_3$ internal

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