THE MECHANISM OF BIOSYNTHESIS OF CITROMYCETIN

S. Gatenbeck and K. Mosbach
Institute of Biochemistry, University of Lund, Sweden

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Most of the acetate-derived phenolic metabolites investigated, belong to a family of compounds whose structure points to a one-chain-polyketo origin. Experimental evidence for this biosynthetic route includes the formation of orsellinic acid (Mosbach, 1961), methylsalicylic acid (Lynen, Tada, 1961) and the anthraquinone islandicin (Gatenbeck, 1962). However, there is a small group of substances including sclerotiorin, monascin, rubropunctatin, rotiorin, fulvic acid and citromycetin whose structure is not consistent with the formation from a linear polyketo chain. Theoretically, they represent either products secondarily derived from metabolites, of the first mentioned type, or originate from two distinct polyketo chains.

The following investigation deals with the biosynthesis of citromycetin with special regard to these two alternatives. The acetate origin of citromycetin has previously been shown (Birch et.al., 1958). The labelling pattern from acetate, however, does not distinguish between formation over a one-chain derived intermediate and condensation of two preformed polyketo structures. A naphtoquinone type compound, whose structure resembles that of citromycetin, could be considered as a possible intermediate in the first case.

The formation of fatty acids and several phenolic compounds is known to occur via the repetitive addition of malonyl-CoA to acetyl-CoA. Acetyl-CoA acts as a chain initator and appears at the methyl terminal end of the chain. The chain grows from the carboxyl end by successive additions of 2 carbon units derived from malonyl-CoA. Experimentally, the administration of 2-C^{14}-malonate should distinguish between the acetyl-CoA and malonyl-CoA derived portions of the chain, provided that the conversion of C^{14}-malonate to malonyl-CoA is faster than the conversion of C^{14}-malonate to acetyl-CoA. Under these circumstance the C^{14}-content of the malonyl-CoA derived portion of the chain will be much higher than that of the acetyl-CoA derived portion.

In the citromycetin there will be one or two starting acetate units depending on a single chain or double chain precursor as outlined in Fig. 1. In the case of alternative A an oxidative opening of the quinone ring is

presumed, analogous to the proposed formation of sulochrin from emodin methylether (Gatenbeck, 1960, Stickings, Mahmoodian, 1962). Thus, only the methyl group in citromycetin will not have full malonate labelling, whereas in alternative B both the methyl and the carboxyl group will show a lower level of radioactivity than the malonate derived portion. In the course of the degradation of malonate-2-^{14}C labelled citromycetin the total radioactivity was determined. Decarboxylation yielded the radioactivity of the carboxyl group. The resulting citromycenone was subsequently oxidized according to the method of Kuhn-Roth and the radioactivity of the resulting acetic acid measured. As follows from the results, listed in Table I, the radioactivity of the carboxyl and methyl groups are 35% and 31% respectively of the malonate level calculated on the basis of the total radioactivity of citromycetin. These findings indicate a two-chain-polyketo origin of citromycetin in the sense of alternative B.

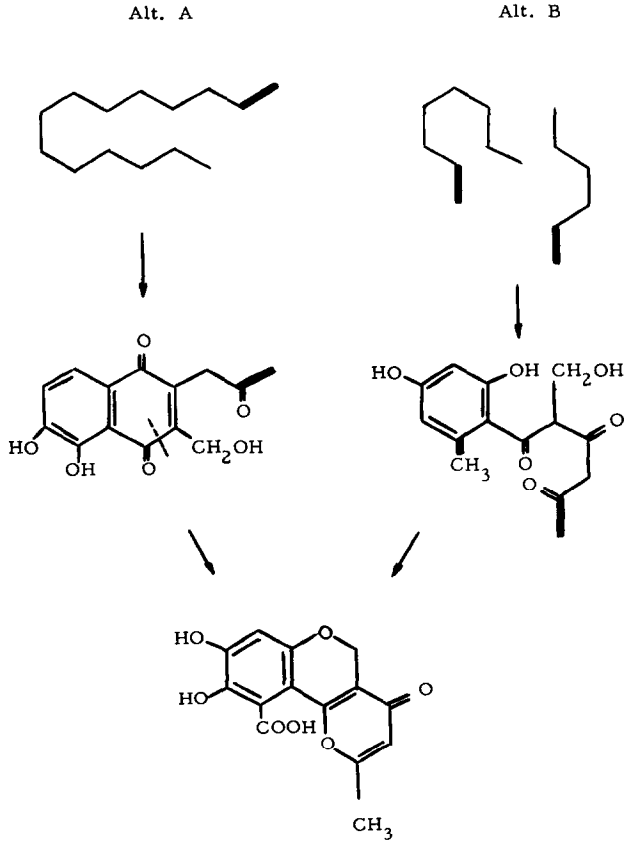

Figure 1

Table I

Malonate-2-^{14}C derived citromycetin

Compound	cpm/mmole x 10^3
citromycetin	155
methyl group	8.6
carboxyl group	9.7
Kuhn-Roth carbon dioxide	147
malonate derived positions	27.3

Experimental.

Culture conditions: A shake culture (250 rpm, 1 inch stroke) of Penicillium frequentans Westling in 500 ml Erlenmeyer flask containing 150 ml of a modified Czapek-Dox medium (Hetherington, Raistrick, 1931) was allowed to grow 4 days, then 50 μc of diethylmalonate-2-^{14}C was added and incubation continued for 2 days.

Isolation: The culture filtrate was extracted with n-butanol, the organic phase evaporated to dryness, and 200 mg of nonlabelled citromycetin added as carrier. The citromycetin was crystallized from water saturated ethylacetate and subsequently recrystallized from an ethanol-water mixture, yield 130 mg, m.p. 283-285 C.

Degradation: 5 mg of the radioactive citromycetin were submitted to wet-combustion and the resulting carbon dioxide trapped as barium carbonate. The remaining 125 mg were decarboxylated to citromycenone by heating in dilute sulphuric acid (Robertson et.al., 1951) and the carbon dioxide collected for radioactive analysis. The citromycenone which crystallized from the reaction mixture was used without further purification for a Kuhn-Roth oxidation. The acetic acid was isolated as sodium acetate after steam distillation. The radioactivity of the methyl group of citromycenone was obtained by combustion of the isolated acetate.

Radioactive analysis: In the degradation series measurements were performed in a liquid scintillation counter with the samples as barium carbonate suspended in a gel of Aerosil in a toluene solution of 2,5-diphenyloxazol.

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