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COMMUNICATION

Studies on Carboxylic Acid Metabolism in a Macrolide-Producing Microorganism Using Carbon-13 Magnetic Resonance

SATOSHI ŌMURA, HIDEO TAKESHIMA, AKIRA NAKAGAWA, NAMI KANEMOTO, AND GABOR LUKACS[1]

Kitasato University and The Kitasato Institute, Minato-ku, Tokyo 108, Japan, and [1]Institut de Chimie des Substances Naturelles du CNRS, 91190 Gif-sur-Yvette, France

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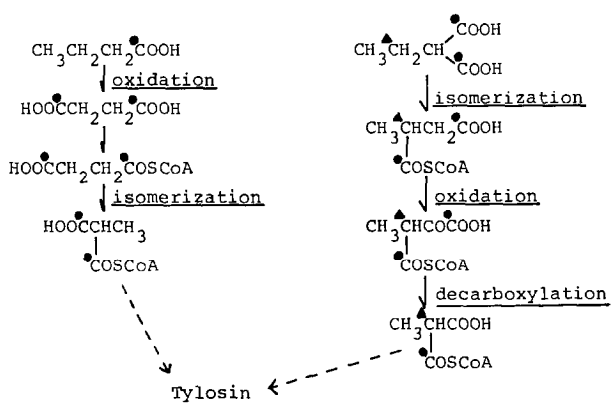
We suggested in a previous paper (*1*) that both butyrate and 2-ethylmalonate are metabolized into methylmalonate and are incorporated into the lactone ring of tylosin, which is a 16-membered macrolide antibiotic produced by *Streptomyces fradiae* C-373. Concerning these results, we proposed metabolic pathways of both precursors into methylmalonate as shown in Scheme 1. In order to confirm our proposal, we have now



SCHEME 1. Proposed pathways of butyric and 2-ethylmalonic acid into methylmalonyl-CoA. Symbols (● and ▲) indicate ^{13}C-enriched carbons.

synthesized [1,4-^{13}C]diethylsuccinate and 2-[1,3,4-^{13}C]ethylmalonic acid using appropriate ^{13}C-labeled starting materials and investigated the mode of incorporation of these ^{13}C-labeled precursors into the lactone ring, by reference (*2*) to a systematic ^{13}C nmr study on 16-membered macrolide antibiotics. In this communication, we report that both [1,4-^{13}C]diethylsuccinate and 2-[1,3,4-^{13}C]ethylmalonic acid are incorporated into the aglycone of tylosin.

[1,4-^{13}]Succinic acid (90 atom% C-13), [1,^{13}C]ethyliodide (90 atom% C-13), and [1,3-^{13}C]diethylmalonate (90 atom% C-13) were purchased from MSD (Japan) Co., Ltd. Strains of *Streptomyces fradiae* C-373 were a gift of Dr. L. E. Day of Eli Lilly and

Co. [1,4-^{13}C]Diethylsuccinate was prepared by esterification of [1,4-^{13}C]succinic acid with absolute ethanol in the presence of a small amount of concentrated H_2SO_4. 2-[1,3,4-^{13}C]Ethylmalonic acid was obtained by [1-^{13}C]ethyliodide alkylation of [1,3-^{13}C]diethylmalonate, followed by hydrolysis of the ester. The preparation of ^{13}C-labeled tylosin was carried out by a method similar to that previously reported (*1*) except that the precursors added were replaced by the newly synthesized compounds. ^{13}C nmr spectra were obtained with a Bruker WP-60 FT nmr spectrometer operating at 15.08 MHz in $CDCl_3$. ^{13}C nmr shifts are expressed in parts per million downfield from internal tetramethylsilane ($\delta = 0$).

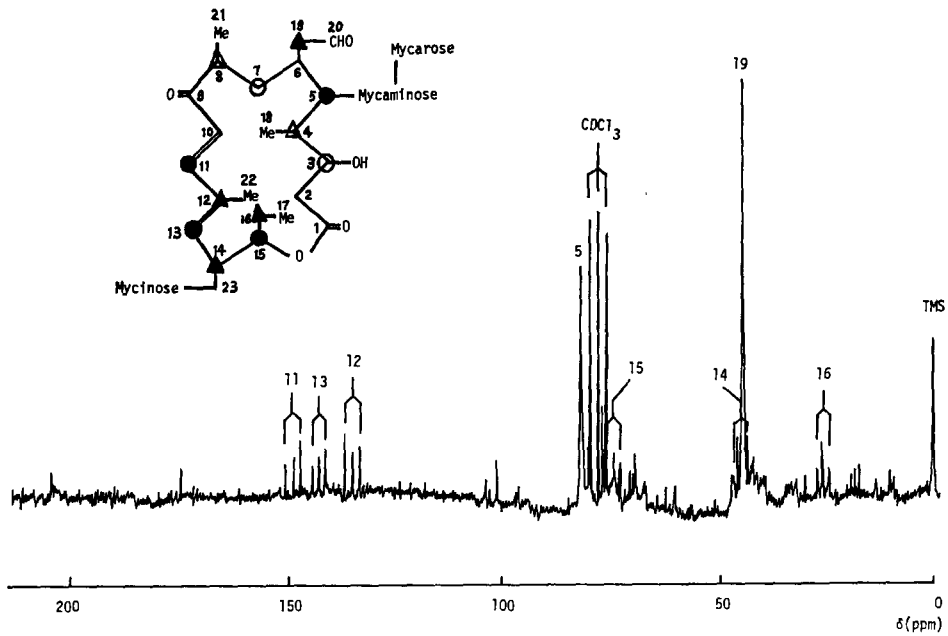


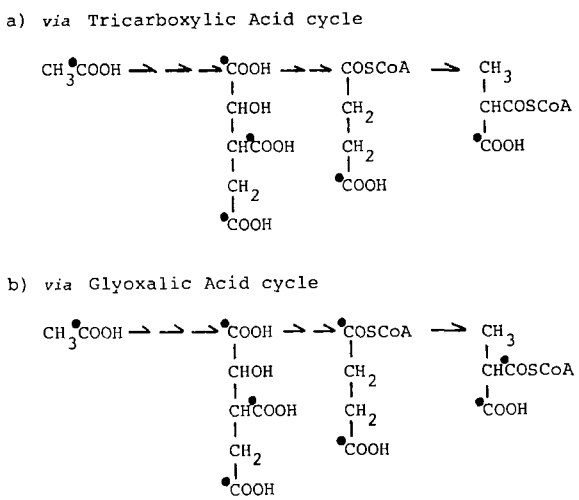
FIG. 1. Carbon-13 nmr spectrum (15.08 MHz) of tylosin which was prepared by the addition of 2-[1,3,4-^{13}C]ethylmalonic acid to the culture media. Concentration is 6.4 mg/ml in $CDCl_3$, and the spectrum is the result of 166,000 accumulations. The symbols (● and ▲) in the structure of tylosin indicate ^{13}C-enriched carbons derived from 2-[1,3,4-^{13}C]ethylmalonic acid in Scheme 1. The open symbols (○ and △) indicate the carbons observed as unclear enrichment signals.

Figure 1 features a ^{13}C nmr spectrum of labeled tylosin obtained from the culture supplemented with 2-[1,3,4-^{13}C]ethylmalonic acid. It revealed, as expected, the enrichment of carbons 5 (δ 81.7) and 19 (δ 43.8). In addition to these two carbons, enriched signals of carbons 11 (δ 148.2), 13 (δ 135.0), 12 (δ 143.9), 14 (δ 44.9), 15 (δ 75.3), and 16 (δ 25.6) were observed and each of these signals was accompanied by a doublet arising from vicinal ^{13}C–^{13}C coupling ($^{J}{}^{13}C_{11} - {}^{13}C_{12} = 49.0$ Hz, $^{J}{}^{13}C_{14} - {}^{13}C_{15} = 41.0$ Hz, $^{J}{}^{13}C_{15} - {}^{13}C_{16} = 36.5$ Hz) as shown in Fig. 1. On the basis of this result, it is suggested that 2-[1,3,4-^{13}C]ethylmalonic acid is isomerized to 2-[1,2,4-^{13}C]methylsuccinyl-CoA by methylmalonyl-CoA mutase (EC 5.4.99.2), or another enzyme having a similar action, and then metabolized to [1,2-^{13}C]methylmalonyl CoA by oxidative decarboxylation of the carboxyl group.

When [1,4-^{13}C]diethylsuccinate[1] was added, carbons 11 (δ 148.2), 13 (δ 135.3), and 15 (δ 75.3) were mainly enriched, which are essentially derived from the carboxyl carbons of propionate. This result and previous data (*1*) indicate that butyrate is converted initially to succinate by ω-oxidation and is further isomerized to methylmalonyl-CoA by the enzyme methylmalonyl-CoA mutase via succinyl CoA; and finally, the converter is incorporated into the aglycone of tylosin.

Thus, we were able to elucidate the metabolic pathways of both precursors to methylmalonyl-CoA in the tylosin-producing microorganism.

In our recent report concerning leucomycin biosynthesis (*3*), we showed that all three carbons which originate from propionate or methylmalonate in the lactone ring of leucomycin were derived from the methyl carbon of acetate and not from the carboxyl carbon of acetate. This evidence indicates that the building unit is formed through the tricarboxylic acid cycle via succinyl-CoA and methylmalonyl-CoA. In other words, if the propionate unit were formed through the glyoxalic acid cycle, at least one carbon of the unit should originate from the carboxyl carbon of acetate, as shown in Scheme 2.



SCHEME 2. Methylmalonyl-CoA formation from acetate. Symbol (●) indicates ^{13}C-enriched carbon.

These results and this discussion emphasize the application of ^{13}C nmr not only to the biosynthesis of secondary metabolites but also to the elucidation of metabolic pathways of simple organic compounds which are building units of the metabolites.

ACKNOWLEDGMENT

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[1] The reason for the use of the diethyl ester of succinic acid was an apparent absence of incorporation after the administration of succinic acid during a preliminary experiment.

REFERENCES

1. S. ŌMURA, A. NAKAGAWA, H. TAKESHIMA, J. MIYAZAWA, C. KITAO, F. PIRIOU, AND G. LUKACS, *Tetrahedron Lett.*, 4053 (1975).
2. S. ŌMURA, A. NAKAGAWA, A. NESZMELYI, S. D. GERO, A. M. SEPULCHRE, F. PIRIOU, AND G. LUKACS, *J. Amer. Chem. Soc.* **97**, 4001 (1975).
3. S. ŌMURA, A. NAKAGAWA, H. TAHESHIMA, K. ATSUMI, J. MIYAZAWA, F. PIRIOU, AND G. LUKACS, *J. Amer. Chem. Soc.* **97**, 6600 (1975).