BIOSYNTHETIC STUDIES OF EBELACTONE A AND B
BY $^{13}$C NMR SPECTROMETRY

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Biosynthetic pathways of ebelactone A and B were studied by $^{13}$C NMR spectroscopy. By using $^{13}$C labeled compounds as precursors it was determined that ebelactone A was derived from one molecule of acetic acid and six propionic acids and ebelactone B from one molecule of acetic acid, five propionic acids and one butyric acid.

As reported in previous papers, we isolated two potent esterase inhibitors, ebelactone A and B from the cultured broth of Streptomyces sp. MG7-G1 and their structures were determined to be 3,11-dihydroxy-2,4,6,8,10,12-hexamethyl-9-oxo-6-tetradecenoic 1,3-lactone (1) and 2-ethyl-3,11-dihydroxy-4,6,8,10,12-pentamethyl-9-oxo-6-tetradecenoic 1,3-lactone (2), respectively.

The macrolide antibiotic produced by Streptomyces were biosynthesized from acetic, propionic and butyric acids. Therefore, we thought that ebelactones might be biosynthesized through a similar pathway. In this paper, we report the incorporation of $^{13}$C-labeled compounds to ebelactones, indicating a pathway similar to the one determined for macrolides.

Materials and Methods

Isotope-labeled Compounds
Sodium [1-14C]acetate (2.7 mCi/mmole) and sodium [1-13C]propionate (2.5 mCi/mmole) were purchased from New England Nuclear, U.S.A. Sodium salts of [1-13C]acetate, [1-13C]propionate and [1-13C]butyrate were purchased from Prochem., England. $^{13}$C-Labeled compounds were 90% enriched products.

Assay of Anti-esterase Activity
Inhibition of esterase of hog liver (Sigma Co., U.S.A.) by ebelactones was determined as reported previously.

Incorporation of Radioactivity into Ebelactones
Spores of Streptomyces sp. MG7-G1 grown on a slant culture were inoculated into 100 ml of a medium consisting of 3% glycerol, 2% fish meal and 0.2% CaCO$_3$, in 500-ml Erlenmeyer flask and cultured for 48 hours at 28°C on a rotatory shaker (180 rpm). After inoculation of 2 ml of the seed culture thus prepared, $^{13}$C-labeled acetate (40 µCi) or propionate (80 µCi) was added to other flasks containing the same medium and the shaking culture was continued.

Aliquots (2 ml) were taken from each flask at intervals shown in Fig. 1 and extracted with an equal volume of butyl acetate. Exactly 0.1 ml of each of the butyl acetate layer was applied on a TLC plate.
Fig. 1. Incorporation of [1-14C]acetate and [1-13C]-propionate into ebelactones.

Radioactivity in ebelactones are shown as the total radioactivity in ebelactones containing in one ml of culture broth.

![Radioactivity in ebelactones](image)

Results and Discussion

Incorporation of [1-14C]Acetate and [1-13C]Propionate to Ebelactones

First, in order to determine whether acetic and propionic acids were precursors, we examined the incorporation of radioactivities of 14C-labeled compounds into ebelactones. As shown in Fig. 1, after 12-hour cultivation, the amounts of incorporated radioactivity of [14C]acetic and [14C]propionic acids increased exponentially and reached maximum at 24 hours. Thus, acetic and propionic acids were shown to be precursors and the best timing to add 13C-labeled compounds into the culture medium was 21 to 24 hours for the production of 13C-labeled ebelactones in a high efficiency.

13C NMR Analysis of Ebelactones

The utilization of propionic acid to form the structure of ebelactone A and B was determined from the 13C NMR spectra shown in Fig. 2 and Fig. 3, respectively. The 13C NMR spectra of [13C]ebelactones A and B produced in the [13C]propionate-containing medium were compared with those of ebelactones. In case of ebelactone A (Fig. 2), the peaks of 1-, 3-, 5-, 7-, 9- and 11-C were enriched and those of 3-, 5-, 7-, 9- and 11-C of ebelactone B (Fig. 3) were enriched. Thus, it was shown that 6 or 5 molecules of propionic acid were used for biosynthesis of ebelactones A and B, respectively.

As described above, the carbon chain of ebelactone A from the 1-C to 12-C is synthesized from pro-
pionic acid. As shown in Table 1, the [1-13C]acetate experiment indicated that 13- and 14-C of ebelactone A were derived from acetic acid. [1-13C]Acetate was incorporated with some enrichment into the 1-, 3-, 5-, 7-, 9- and 11-C besides the high incorporation into the 13-C. The [1-13C]butyrate experiment indicated that it is incorporated into each carbon of ebelactone A with about the same enrichment factors.
As shown in Table 2, the two carbons at 13-C and 14-C of ebelactone B were also derived from acetic acid as in the case of ebelactone A. The [1-13C]butyrate experiment demonstrated that the 1-C of ebelactone B was derived from butyric acid. These results confirmed the incorporation pattern of the building units into ebelactone A and B (Fig. 4).

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References


