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

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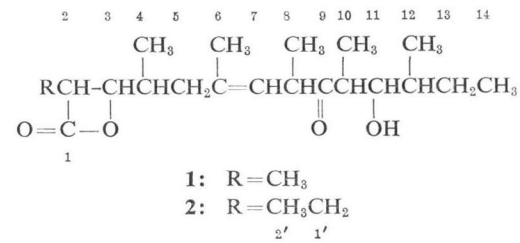
Biosynthetic pathways of ebelactone A and B were studied by ^{13}C NMR spectroscopy. By using ^{13}C labeled compounds as precursors it was determined that ebelactone A was derived from one molecule of acetic acid and six propionic acids and ebelactone B from one molecule of acetic acid, five propionic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

As reported in previous papers,[1,2] 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.[3~5] 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.



1: R = CH_3
2: R = CH_3CH_2

Materials and Methods

Isotope-labeled Compounds

Sodium [1-^{14}C]acetate (2.7 mCi/mmole) and sodium [1-^{14}C]propionate (2.5 mCi/mmole) were purchased from New England Nuclear, U.S.A. Sodium salts of [1-^{13}C]acetate, [1-^{13}C]propionate and [1-^{13}C]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.[1]

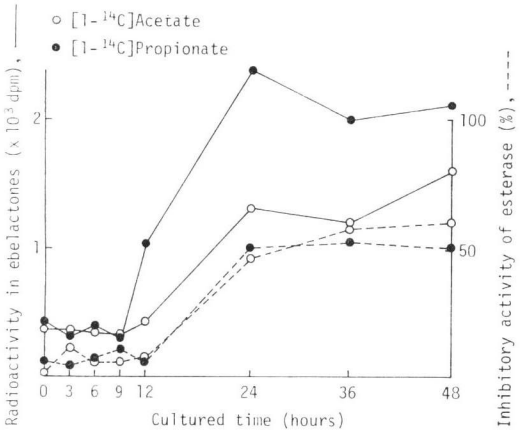
Incorporation of Radioactivity into Ebelactones

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, ^{14}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-^{14}C]acetate and [1-^{14}C]-propionate into ebelactones.

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



of silica gel (E. Merck, Art. 5715) and developed with the solvent system *n*-hexane - chloroform - ethyl acetate (5: 5: 1). Ebelactones were visualized by anisaldehyde-sulfuric acid. The areas of ebelactones were cut out from the plate and transferred into vials for scintillation counting. Five ml of scintillation cocktail (Monophase-40, Packard Co., U.S.A.) was added and the radioactivity of each sample was measured by Aloka-653 liquid scintillation counter.

Preparation of ^{13}C-Labeled Ebelactones

After the strain MG7-G1 was cultured for 21 hours, sodium [1-^{13}C]acetate, [1-^{13}C]propionate or [1-^{13}C]butyrate was added to the medium (100 ml in each flask) at a final concentration of 0.5% and cultured for 3 hours. The contents of 10 flasks were combined and ebelactones A and B were extracted with butyl acetate followed by chromatography on a silica gel column and reversed phase silica gel column chromatography, successively.[1] Usually, 1~2 mg of ^{13}C-labeled ebelactones A and B were obtained from a liter of the cultured broth.

^{13}C NMR Spectrometry

Fourier-transform ^{13}C NMR spectra were taken on a Varian XL-100 NMR spectrometer operating at 25.2 MHz. Each sample was dissolved in 0.25 ml of $CDCl_3$ and was run in a tube with 8 mm diameter at 40°C. The running conditions were as follows: spectral width; 6,016 Hz, pulse width; 15 μseconds and acquisition time; 1.0 second. Accumulations were as follows: 36596 for ebelactone A (1.8 mg) and 44113 for ebelactone B (0.9 mg) labeled with [1-^{13}C]acetate; 25699 for ebelactone A (2.0 mg) and 35365 for ebelactone B (1.0 mg) labeled with [1-^{13}C]propionate; 29613 for ebelactone A (1.0 mg) and 37625 for ebelactone B (2.0 mg) labeled with [1-^{13}C]butyrate.

Results and Discussion

Incorporation of [1-^{14}C]Acetate and [1-^{14}C]Propionate to Ebelactones

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

^{13}C NMR Analysis of Ebelactones

The utilization of propionic acid to form the structure of ebelactone A and B was determined from the ^{13}C NMR spectra shown in Fig. 2 and Fig. 3, respectively. The ^{13}C NMR spectra of [^{13}C]ebelactones A and B produced in the [^{13}C]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-

Fig. 2. ^{13}C NMR spectrum of ebelactone A.

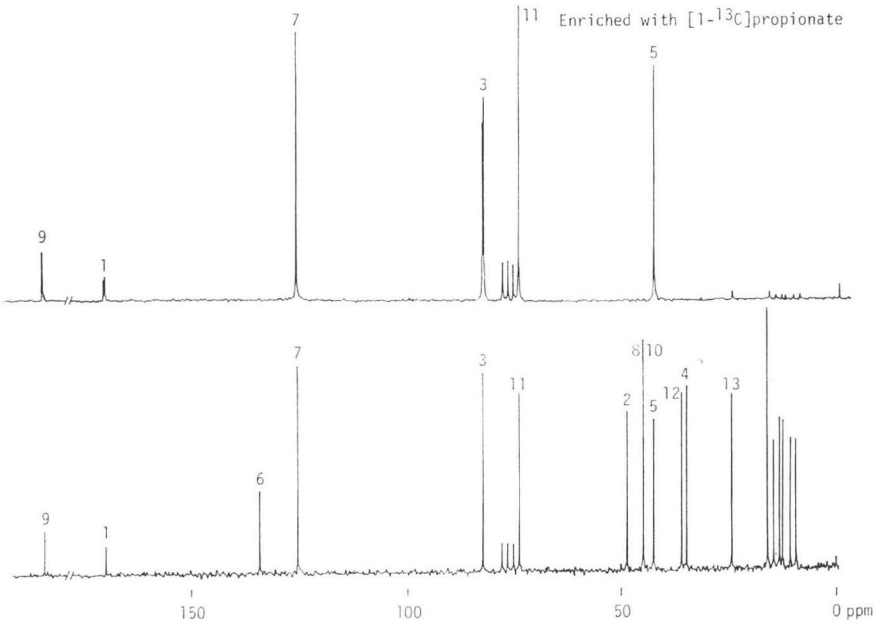
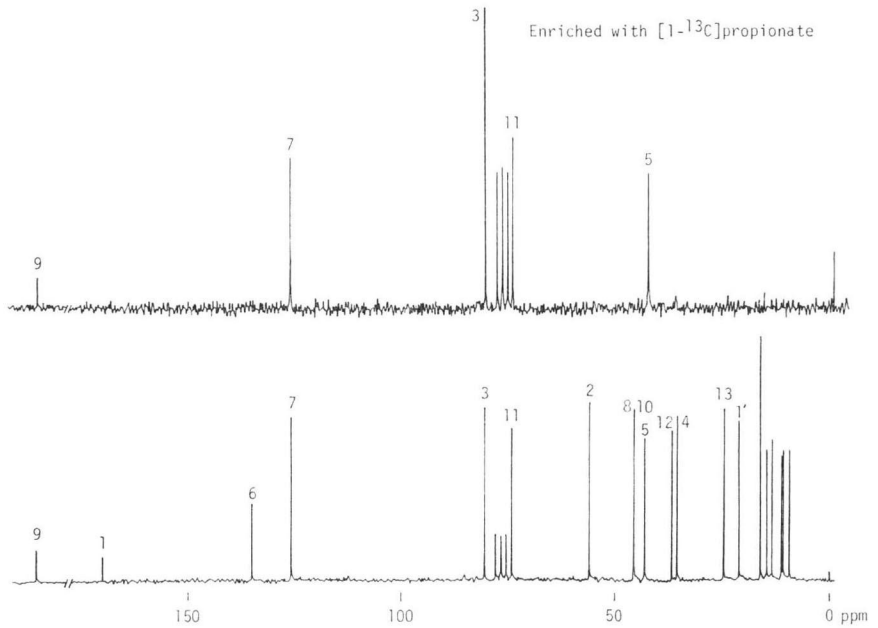


Fig. 3. ^{13}C NMR spectrum of ebelactone B.



pionic acid. As shown in Table 1, the [1-^{13}C]acetate experiment indicated that 13- and 14-C of ebelactone A were derived from acetic acid. [1-^{13}C]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-^{13}C]butyrate experiment indicated that it is incorporated into each carbon of ebelactone A with about the same enrichment factors.

Table 1. ^{13}C NMR spectral data for ebelactone A, including enrichments from labeled precursors.

Carbon No.	δ_c[a]	Multiplicity[b]	Enrichment factor[c]	
			[1-^{13}C]-acetate	[1-^{13}C]-butyrate
1	171.7	s	3.94	—[d]
2	49.1	d	0.81	0.86
3	82.9	d	3.88	0.75
4	35.5	d	0.69	0.68
5	42.9	t	4.88	0.96
6	135.5	s	0.50	—[d]
7	126.5	d	4.06	0.71
8	45.3	d	0.50	0.57
9	217.5	s	3.69	—[d]
10	45.2	d	0.50	0.57
11	74.6	d	4.38	0.93
12	36.6	d	0.56	0.76
13	24.9	t	13.50	0.96
14	13.5	q	0.94	0.82
2-CH_3	12.9	q	0.88	0.79
4-CH_3	10.9	q	0.69	0.82
6-CH_3	16.4	q	} 0.94	} 0.82
8-CH_3	16.4	q		
10-CH_3	9.5	q	1.00	1.00
12-CH_3	14.9	q	0.88	0.82

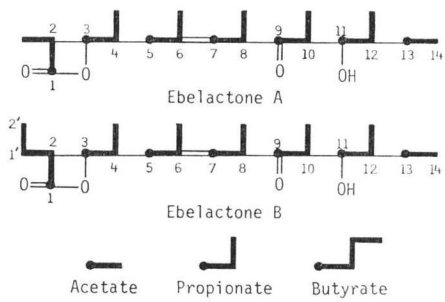
[a] Chemical shifts are downfield from internal Me_4Si in $CDCl_3$.
[b] Multiplicities in the off-resonance decoupling; s, singlet; d, doublet; t, triplet; q, quartet.
[c] Intensity of each peak in the labeled ebelactone divided by that of the corresponding peak in the unlabeled ebelactone, normalized to give a ratio of 1.00 for the peak of 10-CH_3.
[d] Signals for these carbons, which were weak in the unlabeled ebelactone, were lost in the noise.

Table 2. ^{13}C NMR spectral data for ebelactone B, including enrichments from labeled precursors.

Carbon No.	δ_c[a]	Multiplicity[b]	Enrichment factor[c]	
			[1-^{13}C]-acetate	[1-^{13}C]-butyrate
1	171.7	s	—[d]	8.24
2	56.0	d	1.00	—[d]
3	81.1	d	6.17	0.72
4	35.5	d	0.92	0.64
5	42.9	t	6.66	1.16
6	135.5	s	—[d]	—[d]
7	126.5	d	7.17	1.32
8	45.4	d	1.00	0.68
9	217.5	s	7.17	—[d]
10	45.2	d	1.00	0.68
11	74.6	d	7.25	1.72
12	36.6	d	1.00	0.80
13	24.9	t	17.80	1.00
14	13.7	q	1.08	0.84
1′	21.4	t	2.41	0.64
2′	11.4	q	1.16	—[d]
4-CH_3	10.9	q	1.08	0.80
6-CH_3	16.4	q	} 1.08	} 0.76
8-CH_3	16.4	q		
10-CH_3	9.5	q	1.00	1.00
12-CH_3	14.9	q	0.83	0.76

[a], [b], [c], [d]: See Table 1.

Fig. 4. Biosynthesis of ebelactone A and B.



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-^{13}C]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

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