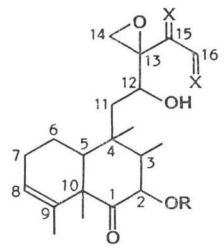
BIOSYNTHETIC STUDIES OF TERPENTECIN

Sir:

In a previous paper[1] we reported the structure of terpentecin (**1**)[2], a diterpenoid antitumor antibiotic produced by the strain MF730-N6 classified as a *Kitasatosporia* species. Here we wish to report a labeling study of terpentecin with [5-^{13}C]mevalonolactone and sodium [2-^{13}C]-acetate. The result supported the structure previously reported[1].

A 500-ml Erlenmeyer flask containing 110 ml of the seed medium (pH 7.2) composed of glucose 1% and yeast extract 1% was inoculated with strain MF730-N6 and the culture was grown at 27°C for 24 hours on a rotary shaker. A 3-ml portion of the seed culture thus prepared was used to inoculate each 500-ml Sakaguchi

Fig. 1. The structure of terpentecin (**1**) and it's derivative (**2**).



Terpentecin (**1**) X = O R = H

2 X = $CHCOOCH_3$ R = *p*BrBz

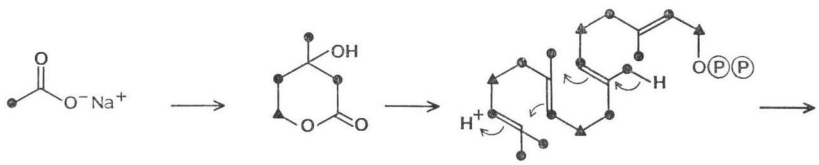
Table 1. Chemical shifts and incorporation of [2-^{13}C]acetate and [5-^{13}C]mevalonolactone.

Carbon atom	Chemical shift[a] (ppm)	Normalized peak height[b]	
		[2-^{13}C]-Acetate	[5-^{13}C]-Mevalono-lactone
1	207.8	1.4	4.6
2	77.1	3.9	1.5
3	41.4	1.7	1.4
4	38.9	2.0	1.5
5	41.1	2.1	1.3
6	19.3	3.0	1.6
7	26.1	1.7	4.1
8	124.4	2.9	2.5
9	137.8	2.1	1.7
10	51.8	2.3	1.7
11	40.2	1.4	5.2
12	67.7	3.7	1.2
13	63.8	1.0	1.0
14	49.1	2.7	2.0
15	146.4	1.9	2.2
16	136.8	1.6	5.4
3-CH_3	11.2	2.4	2.3
4-CH_3	24.2	2.4	2.7
9-CH_3	20.6	2.8	2.6
10-CH_3	23.8	2.8	2.9

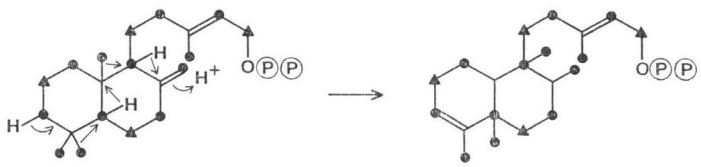
[a] Measured in $CDCl_3$ solution, in ppm down field from internal TMS. Spectra were recorded on a Jeol GX-400 spectrometer. Signal assignment were reported in ref 1.

[b] Intensity of each peak was normalized based on the peak intensity at the C-13 position as standard.

Scheme 1.



Mevalonolactone Geranyl-geranyl pyrophosphate



Pyrophosphate of clerodane skeleton

flask containing 125 ml of medium (glucose 0.04%, galactose 0.08%, maltose 0.08%, dextrin 0.16%, Bacto-soytone 0.08% and ammonium sulfate 0.03%, pH 7.0). Fermentation was carried out for 28 hours at 27°C. ^{13}C-Labeled precursors were added after 0 and 6 hours incubation. The dose per flask was 2 mg for [5-^{13}C]mevalonolactone and 10 mg for [2-^{13}C]-acetate. The ^{13}C-labeled terpentecin was isolated from the culture filtrate and converted chemically to the stable form **2** as reported in a previous paper[1] (Fig. 1).

As shown in the ^{13}C NMR spectrum (Table 1), the signal intensities of the carbon atoms C-1, -7, -11 and -16 of [5-^{13}C]mevalonolactone-labeled compound **2** were increased approximately 5-fold. This result indicates that terpentecin (which has a clerodane skeleton[3]) is biosynthesized from 4 molecules of mevalonate *via* geranyl-geranyl pyrophosphate[4] as illustrated in Scheme 1.

The incorporation rates of [5-^{14}C]mevalonate and [*U*-^{14}C]acetate were 1.8% and 0.1% (data is not shown). Probably due to the low incorporation, the values of normalized peak height in the ^{13}C NMR spectrum of the terpentecin derivative (**2**) ^{13}C-enriched from ^{13}C-acetate should include a rather large error variation. However, the ^{13}C NMR spectrum of [2-^{13}C]-acetate-derived terpentecin derivative (**2**) showed twelve enhanced peaks corresponding to C-2, -4, -6, -8, -10, -12, -14, -15, 3-CH_3, 4-CH_3, 9-CH_3 and 10-CH_3. This labeling pattern also supported the biosynthetic route, *via* mevalonate and geranyl-geranyl pyrophosphate.

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