Biosynthesis of Albocycline: Origin of the Carbon Skeleton

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The antibiotics cineromycin B (**2**)[1] and albocycline (**3**)[2] are the main components of a family of 14-membered macrolides, which exhibit striking biological activities in different areas. In contrast to classical macrolide antibiotics as erythromycin or tylosin[3], **2** and **3** do not contain sugar moieties, which are normally necessary to activate the aglycon. In this sense, **2** and **3** are unusual macrolides. Their biological activity is bound to a skeleton, which seems to be a nature-optimized structure, because variation under preservation of the activity are very limited[4~7]. This paper deals with the biogenetic origin of the carbon skeleton of albocycline (**3**).

Because of the structural similarities of **3** with other macrolides, we assume a polyketide pathway with acetate and propionate units as building blocks[8,9]. In order to prove this, we fed sodium [1-^{13}C]acetate and sodium [1-^{13}C]propionate to the growing cultures of *Streptomyces* sp. (strain Lu 7285), which recently has been shown as a producer of albocycline (**3**) and 2,3-dihydroalbocycline (**5**)[7].

Experiments were carried out using cultures of strain Lu 7285, grown at 28°C for 7 days in 250 ml Erlenmeyer flasks filled with 50 ml of medium (degreased soybean meal 2%, mannitol 2%, pH adjusted to 7.2). Pulse feeding experiments with both, [1-^{13}C]acetate and [1-^{13}C]propionate were carried out in a 1 liter-fermentor using 800 ml of the same medium inoculated with 2 × 50 ml of the above cultures. 12.2 mmol/liter of labeled acetate and 8.3 mmol/liter of labeled propionate, respectively, dissolved in 130 ml of sterile water, were added between 19 and 40 hours after inoculation. The isolation of **3** and **5** was performed after 96 hours by separating the mycelium by centrifugation and extracting the culture filtrate with a comparable volume of ethyl acetate. The dried extracts (0.3 g of a dark brown oil) were purified as described recently[7] yielding 40.2 mg/liter of **3** and

Fig. 1. Biosynthesis of the carbon skeleton of albocycline related macrolides and proposed pathway of the late biosynthesis. (DCBS: 4-Deoxycineromycin B synthase).

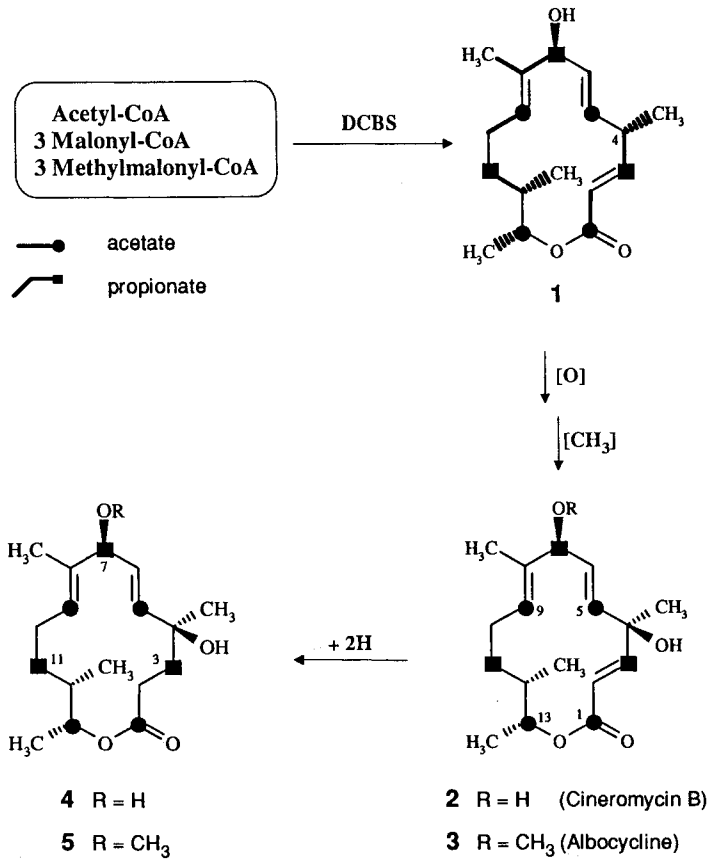


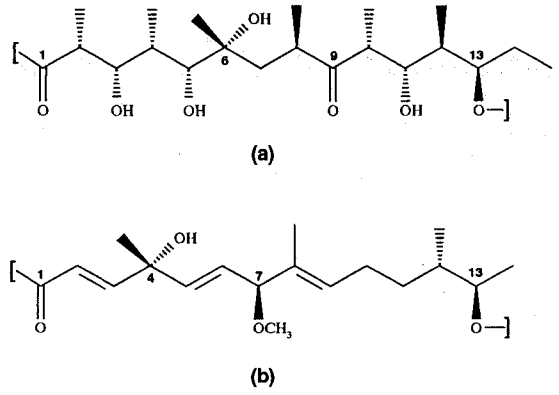
Table 1. Chemical shifts and specific incorporations (standarized to 7-OCH_3 signal intensity) of albocycline (**3**) and 2,3-dihydroalbocycline (**5**) after feeding with sodium [^{13}C]acetate and sodium [^{13}C]propionate ($CDCl_3$, 125.7 MHz).

C-Atom	δ_C (ppm)		Specific incorporation			
			[1-^{13}C]Acetate		[1-^{13}C]Propionate	
	3	**5**	**3**	**5**	**3**	**5**
1	166.2	173.6	**6.18**	**7.72**	0.16	0.07
2	115.4	30.4	0.04	−0.13	−0.48	−0.19
3	154.7	37.0	2.78	2.84	**53.37**	**72.52**
4	73.2	72.2	−0.06	0.21	−0.47	−0.38
5	135.9	137.3	**8.39**	**13.00**	0.10	−0.18
6	130.8	128.7	0.06	−0.26	−0.55	−0.40
7	84.8	87.8	1.76	1.41	**55.87**	**57.84**
8	136.5	136.4	0.16	−0.13	0.59	−0.55
9	129.1	127.6	**6.45**	**11.95**	−0.28	−0.17
10	24.7	22.6	−0.21	−0.22	−0.53	−0.55
11	34.2	31.8	1.95	2.88	**55.77**	**62.85**
12	39.1	35.4	−0.15	−0.15	−0.53	−0.49
13	75.5	72.5	**6.97**	**9.25**	0.23	0.36
7-OCH_3	56.9	55.5	0.00	−0.00	0.00	−0.00
4-CH_3	27.0	30.4	0.07	−0.23	0.09	−0.19
8-CH_3	13.9	11.2	−0.08	0.27	−0.17	0.16
12-CH_3	15.7	15.15	0.04	−0.12	0.12	0.39
13-CH_3	17.8	15.11	−0.03	−0.00	−0.25	0.32

23.1 mg/liter of **5** from the [1-^{13}C]acetate and 37.1/18.3 mg/liter from the [1-^{13}C]propionate experiment, respectively.

The isolated samples of **3** and **5** were analyzed by ^{13}C-NMR spectroscopy resulting in the labeling pattern depicted in Table 1 and shown in formulae **3** and **5**. As expected, the feeding of [1-^{13}C]acetate resulted in signal enhancements of C-1, C-5, C-9 and C-13, while C-3, C-7 and C-11 were labeled by [1-^{13}C]propionate. L-[Methyl-^{13}C]methionine as precursor led to an inhibition of the growth of our strain and of the albocycline production as well. Thus, the carbon skeleton of albocycline (**3**) is derived from four acetate and three propionate units. Following the usual polyketide pathway for macrolides we assume 4-deoxycineromycin B (**1**) as the intermediate, which is released from the polyketide synthase (PKS) by a thioesterase[10]. **1** is further modified by successive post-polyketide steps as hydroxylation at C-4, resulting in cineromycin B (**2**) and *O*-methylation, resulting in albocycline (**3**) (Fig. 1). 2,3-Dihydroalbocycline (**5**) seems to be a product of the late biosynthesis, because it appears after **3** during the time course of the fermentation. The results confirm the large variety of polyketide synthases in using acetate and propionate building blocks. The alternating sequence (APAPAPA) of the albocycline skeleton is a very unusual one. Furthermore the presence and position of three not conjugated double bonds are

Fig. 2. Polyketide frameworks of erythronolide B (a) and albocycline (b).



unique within 14-membered macrolides. This points to a striking dehydratase activity within the subunits of the belonging PKS. For the hydroxy group at C-7 of **1** we assume a genuine PKS activity, because the keto derivative of **1** appears only as minor component of the cineromycin complex[6] and never has been found as a co-metabolite of albocycline producers[5,7]. The stereochemistry at C-13 of **3** corresponds with that normally found in the starter unit of polyketide macrolactones[10,11]. The other centers of chirality are not comparable to that of the classical macrolides *e.g.*

erythronolide B (Fig. 2). The described special structure elements make albocycline or cineromycin B producer strains to suited candidates for genetic engeneering[12,13]. They may be donors of selected biosynthesis gene modules, which encodes for an acetate specifity, a prefered generation of double bonds and/or other stereochemistry features within a parent modular PKS[12~14].

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