BIOSYNTHESIS OF ANTIBIOTIC 1233A (F-244) AND PREPARATION OF [^{14}C]1233A

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The biosynthesis of antibiotic 1233A (F-244) was studied by feeding ^{13}C-labeled precursors to the producing organism, *Scopulariopsis* sp. F-244. ^{13}C NMR spectroscopy established that 1233A is derived from 4 methionines and 7 acetates. Seven acetates are condensed to form a hexaketide and 4 methyl residues from methionine are introduced into the main skeleton. The β-lactone is derived from the α-carboxylic acid of the hexaketide. Since methionine was efficiently incorporated into 1233A, radiolabeled 1233A was prepared biosynthetically by feeding [^{14}C]methionine to the producer. As a result, [^{14}C]1233A was obtained with high specific radioactivity (27.2 μCi/μmol).

Antibiotic 1233A (F-244, L-659,699) isolated from *Scopulariopsis* sp. F-244 is a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase.[1~3] The structure was determined by CHAING *et al.* as shown in Fig. 1.[4] Although 1233A is expected to be synthesized *via* the polyketide pathway,[5] the biosynthetic pathway has not been studied. Our interests were; 1) to determine which end (C-1 and -2 unit of the lactone moiety or C-13 and -14 unit containing the free carboxylic acid) is the primer acetate and 2) what is the origin of methyl (C-8′, -10′ and -12′) and hydroxymethyl (C-2′) side chains of 1233A. To clarify the biosynthesis scheme, we prepared ^{13}C-enriched 1233A by feeding ^{13}C-labeled precursors to cultures of *Scopulariopsis* sp. F-244. This paper describes the spectroscopic analysis of ^{13}C-labeled 1233A and the preparation of radiolabeled 1233A.

Materials and Methods

Isotope-labeled Compounds

L-[*Methyl*-^{14}C]methionine (57.0 μCi/μmol) was purchased from New England Nuclear, U.S.A. Sodium [1-^{13}C]acetate, sodium [2-^{13}C]acetate, sodium [1,2-$^{13}C_2$]acetate and L-[*methyl*-^{13}C]methionine were purchased from ISOTEC Inc., U.S.A.

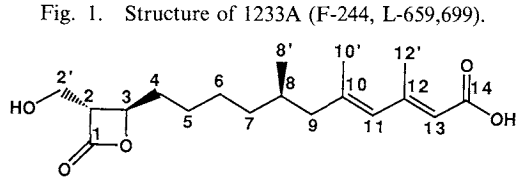
Microorgansim

Scopulariopsis sp. F-244 was used to produce 1233A.

Cultivation

Spores of *Scopulariopsis* sp. F-244 grown on yeast extract-soluble starch (YpSs) agar were inoculated into a culture tube (2 × 20 cm) containing 10 ml of seed medium (glucose 2%, meat extract 0.5%, peptone 0.5%, dried yeast cells 0.3% and NaCl 0.5%, w/v, pH 7.0). The tube was incubated at 27°C for 2 days with reciprocal shaking (280 strokes/minute). To produce ^{13}C-labeled 1233A, the seed culture (1.0 ml) was transferred into a 500-ml Erlenmeyer flask containing 100 ml of production medium (glycerol 2%, glucose 1%, CH_3COONH_4 0.5%, K_2HPO_4 0.1%, KCl 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05% and agar 0.1%, w/v, pH 6.0). Sodium [1-^{13}C]acetate, sodium

Fig. 1. Structure of 1233A (F-244, L-659,699).



[2-^{13}C]acetate, sodium [1,2-$^{13}C_2$]acetate (final concentration 0.1%, w/v) and L-[*methyl*-^{13}C]methionine (final concentration 0.025%, w/v) were fed at the 78th hour of cultivation. Feeding the labeled sodium acetates and methionine was stopped at the 93rd and 120th hour, respectively. To produce [^{14}C]1233A, the seed culture (0.3 ml) was inoculated into a 100-ml Erlenmeyer flask containing 30 ml of the production medium. [^{14}C]Methionine (0.25 mCi) was added to the culture medium twice, at the 130th and 142nd hours of cultivation. The fermentation was stopped at the 150th hour. Labeled 1233A was isolated as reported previously.[6] In brief, the whole broth was extracted with ethyl acetate under the acidic condition and labeled 1233A was purified by HPLC using an ODS column.

Measurement of Production and Radioactivity of [^{14}C]1233A

To 100 μl of culture broth withdrawn was added 100 μl of ethanol and the mixture was voltexed. After centrifugation, a portion of the supernatant (50 μl) was directly injected into HPLC to determine the amount of 1233A. The conditions of HPLC were as follows: Apparatus; Waters 600E system, column; Waters μBondasphere 5 μC_{18}-100A (3.9 × 150 mm), eluant; 45% CH_3CN-0.05% CH_3COOH, flow rate; 1.0 ml/minute, detection; UV at 270 nm. 1233A was eluted at a retention time of 6.6 minutes. To measure the specific radioactivity of [^{14}C]1233A, a portion of the [^{14}C]1233A fraction purified by HPLC was transferred into a scintillation vial. Thereafter 3 ml of a scintillation solvent (ACSII, Amersham Corp.) was added, and radioactivity of 1233A was measured in a scintillation spectrometer (Aloka).

Results

Biosynthesis of Antibiotic 1233A

The ^{13}C NMR spectrum of ^{13}C-enriched 1233A produced in the sodium [1-^{13}C]acetate-containing

Fig. 2. ^{13}C NMR spectra of 1233A.

(A) Natural 1233A, and ^{13}C-enriched 1233A obtained in (B) sodium [1-^{13}C]acetate and (C) [*methyl*-^{13}C]methionine feeding experiments.

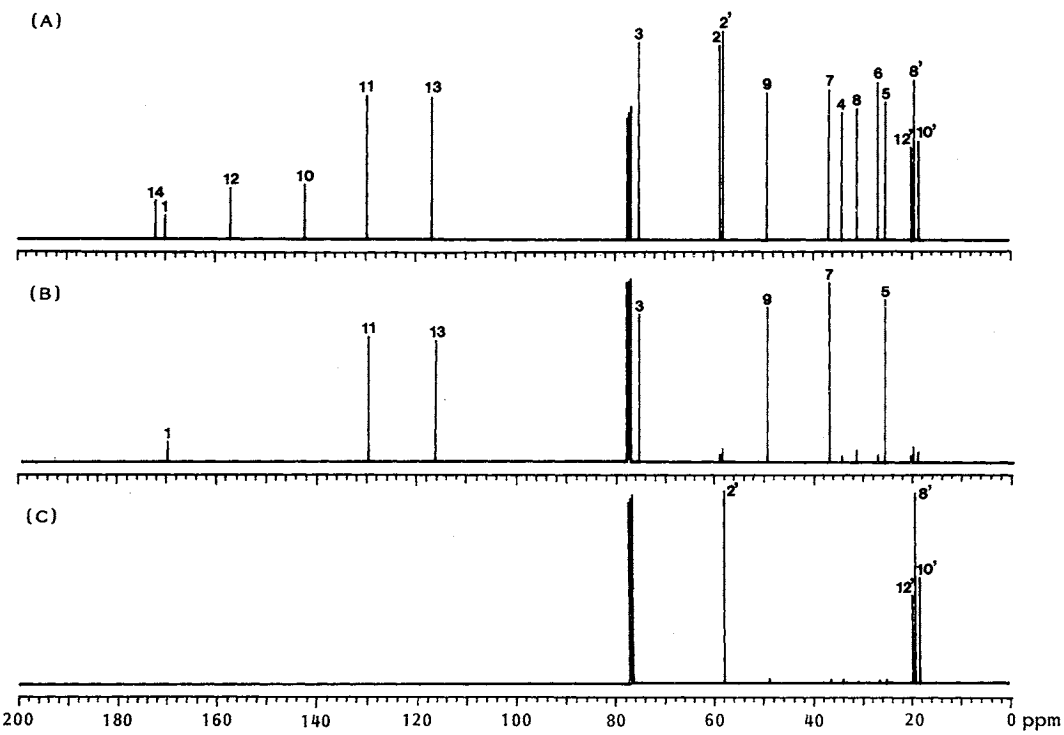


Table 1. ^{13}C NMR chemical shifts, enrichment ratio of 1233A derived from ^{13}C-single-labeled precursors and the $J_{C\text{-}C}$ of sodium [1,2-$^{13}C_2$]acetate-labeled 1233A.

Carbon	^{13}C Chemical shift ppm[a]	Mult.[b]	Enrichment ratio[d] of 1233A derived from			[1,2-$^{13}C_2$]AcONa[c] $J_{C\text{-}C}$ (Hz)
			[1-^{13}C]AcONa[c]	[2-^{13}C]AcONa[c]	L-[*Me*-^{13}C]Met[c]	
C-1	169.96	s	6.57	—[e]	—[e]	41.52
C-2	58.60	d	0.36	5.68	—[e]	41.53
C-3	75.01	d	6.76	0.68	—[e]	38.70
C-4	33.98	t	0.47	9.48	0.93	38.69
C-5	25.17	t	10.40	0.71	1.00	34.65
C-6	26.60	t	0.40	6.19	0.75	34.89
C-7	36.56	t	10.76	0.95	0.71	34.89
C-8	30.91	d	0.82	7.88	0.51	34.89
C-9	48.98	t	9.44	0.63	0.77	41.89
C-10	142.09	s	—[e]	5.67	—[e]	41.90
C-11	129.50	d	7.85	0.54	—[e]	52.34
C-12	157.05	s	—[e]	4.70	—[e]	50.87
C-13	116.73	d	7.60	0.38	—[e]	72.73
C-14	172.04	s	—[e]	2.27	—[e]	75.19
C-2′	57.94	t	0.61	0.56	29.87	s[f]
C-8′	19.39	q	0.82	0.90	30.60	s[f]
C-10′	18.50	q	1.00	1.00	25.33	s[f]
C-12′	19.98	q	0.92	0.71	23.60	s[f]

[a] Each sample was dissolved in $CDCl_3$. Chemical shifts are shown with reference to $CDCl_3$ as 77.01 ppm.
[b] Multiplicities determined from DEPT spectrum.
[c] AcONa = sodium acetate; Met = methionine.
[d] Enrichment ratio was relative to the 10′-CH_3 signal ([1-^{13}C]- and [2-^{13}C]acetates) as 1.0, and to the C-5 signal ([*Me*-^{13}C]Met) as 1.0, respectively.
[e] Signals were lost in the noise.
[f] Signal was a singlet, so the carbon had no coupling with the others.

Fig. 3. Biosynthesis of 1233A.

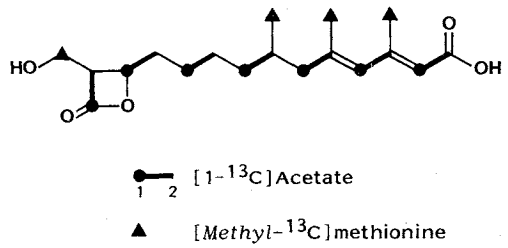
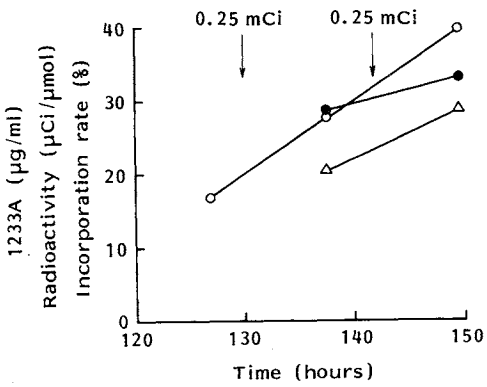


Fig. 4. Time course of [^{14}C]1233A production, specific radioactivity and incorporation rate of [^{14}C]methionine into 1233A.

○ 1233A, ● incorporation rate, △ radioactivity.



medium was compared with that of natural 1233A (Fig. 2). The 7 signals of C-1, -3, -5, -7, -9, -11 and -13 were enhanced (Table 1), indicating the main skeleton of 1233A is a polyketide from 7 acetate units. The conclusion was corroborated by the enhanced 7 alternative signals (C-2, -4, -6, -8, -10, -12 and -14) of ^{13}C-1233A from the [2-^{13}C]acetate feeding experiment and by the 7 pairs of C-C coupling constant from the [1,2-$^{13}C_2$]acetate feeding experiment (Table 1). In the ^{13}C-enriched 1233A obtained by feeding [*methyl*-^{13}C]methionine, the signals of C-2′, -8′, -10′ and -12′ were enhanced (Fig. 2 and Table 1). This indicates that the main skeleton of 1233A is derived from 7 acetate units and that the side chains are

derived from the methyl residue of 4 methionines (Fig. 3).

Preparation of [^{14}C]1233A

From the enrichment ratios shown in Table 1, it is expected that the methyl residue of [*methyl*-^{13}C]methionine can be incorporated very efficiently into the side chains (C-2', -8', -10' and -12') of 1233A. To prepare 1233A with high specific radioactivity, [*methyl*-^{14}C]methionine was used as a precursor. As shown in Fig. 4, 0.25 mCi of [^{14}C]methionine was fed to the producing organism twice, on the 130th and the 142nd hours of cultivation. At the 150th hour, the culture broth was extracted with EtOAc to isolate [^{14}C]1233A. The production of 1233A was increased gradually from 17 μg/ml at the 127th hour to 40 μg/ml at the 150th hour of cultivation. Eventually, [^{14}C]1233A with high specific radioactivity (27.2 μCi/μmol, 0.62 mg) was obtained. The incorporation rate of [^{14}C]methionine into [^{14}C]1233A was very high (31.4%).

Discussion

The ^{13}C-labeled precursor feeding experiments demonstrated that antibiotic 1233A is composed of 7 acetates and 4 methionines. Seven acetate units appeared to be condensed in a "head-to-tail" fashion to form a hexaketide and the side chains of methyl residues were introduced from methionine at the C-2', -8', -10' and -12' carbons. Simultaneously, the following reactions may be involved; ω-oxidation of the head methyl residue of the hexaketide to form carboxylic acid, β-lactone formation of the α-carboxylic acid tail, and oxidation of the 2'-methyl residue. UOTANI *et al.*[7] reported the biosynthesis of ebelactones, esterase inhibitors. Ebelactones isolated from *Streptomyces* sp. were structurally related to 1233A although the absolute configuration (2*S*, 3*S*) of the β-lactone moiety is opposite to that of 1233A (2*R*, 3*R*).[8] They demonstrated that all the methyl residues of ebelactones are derived from propionate. On the other hand, all the methyl residues of 1233A were found to be derived from methionine. This coincides with the evidence that the methyl side chains of fungal metabolites are generally derived from methionine.

High incorporation of [*methyl*-^{13}C]methionine into the side methyl residues (C-2', -8', -10' and -12') of 1233A (Table 1), led to the preparation of [^{14}C]1233A with high specific radioactivity. There have been debates on the mechanism of action of 1233A on HMG-CoA synthase. Our preliminary data indicated that 1233A inhibits the enzyme irreversibly.[2,9] GREENSPAN *et al.*[3] showed the reversible inhibition by 1233A. On the contrary, MAYER *et al.*[10] proposed the irreversible inhibition of 1233A from kinetic analysis. The purpose of our preparing [^{14}C]1233A was to clarify this problem. Now, biosynthetically-prepared [^{14}C]1233A enables us to study the direct interaction between HMG-CoA synthase and [^{14}C]1233A. We have concluded that [^{14}C]1233A specifically binds HMG-CoA synthase covalently. The details will be published elsehwere.[11]

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