SELECTION OF A SPECIFICALLY BLOCKED MUTANT OF *STREPTOMYCES CINNAMONENSIS*: ISOLATION AND SYNTHESIS OF 26-DEOXYMONENSIN A

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Streptomyces cinnamonensis produces the polyether ionophore antibiotic monensin A. Following a single round of mutagenesis by UV light, a derivative of this strain has been isolated, which secretes a new metabolite identified as 26-deoxymonensin A (**3**). The structural elucidation of the new metabolite followed from a spectroscopic analysis, and its identity was proven conclusively following a comparison to 26-deoxymonensin A (**3**) obtained synthetically from monensin A. The preparation of labelled forms of **3** is described, together with incorporation experiments using the parent strain of *S. cinnamonensis*. Only very low levels of incorporation of **3** into monensin A were observed.

Monensin A (**1**) is an important polyether ionophore antibiotic, produced[1] by *Streptomyces cinnamonensis*. The biosynthesis of this antibiotic has attracted a great deal of interest[2~5], following proposals[3,4] that the cyclic ether groups in monensin A might arise by the novel mechanism shown in Scheme 1, proceeding *via* a cascade of cyclisation steps on a triepoxide intermediate. This route is supported by the results of isotope labelling experiments[3~5] that define the biosynthetic origins of the carbon and oxygen atoms in monensin A, and similar experiments with other polyethers, including narasin[6], lenoremycin[7], lasalocid A[8], ICI 139603[9] and maduramicin[10], strengthen a belief that this very appealing concept may be applied generally[11] to account for the formation of cyclic ether rings in many of the other known members of this family of secondary metabolites.

In order to further characterize the pathway to the monensins A (**1**) and B (**2**) in *S. cinnamonensis*, we have generated mutants blocked in monensin production, and describe here the isolation of one such mutant that accumulates mainly 26-deoxymonensin A (**3**); the mutant must carry a lesion in the hydroxylation step occuring at the terminal C-26 position. The structure assigned to **3** has been confirmed by direct comparison with a sample of 26-deoxymonensin A prepared chemically from monensin A.

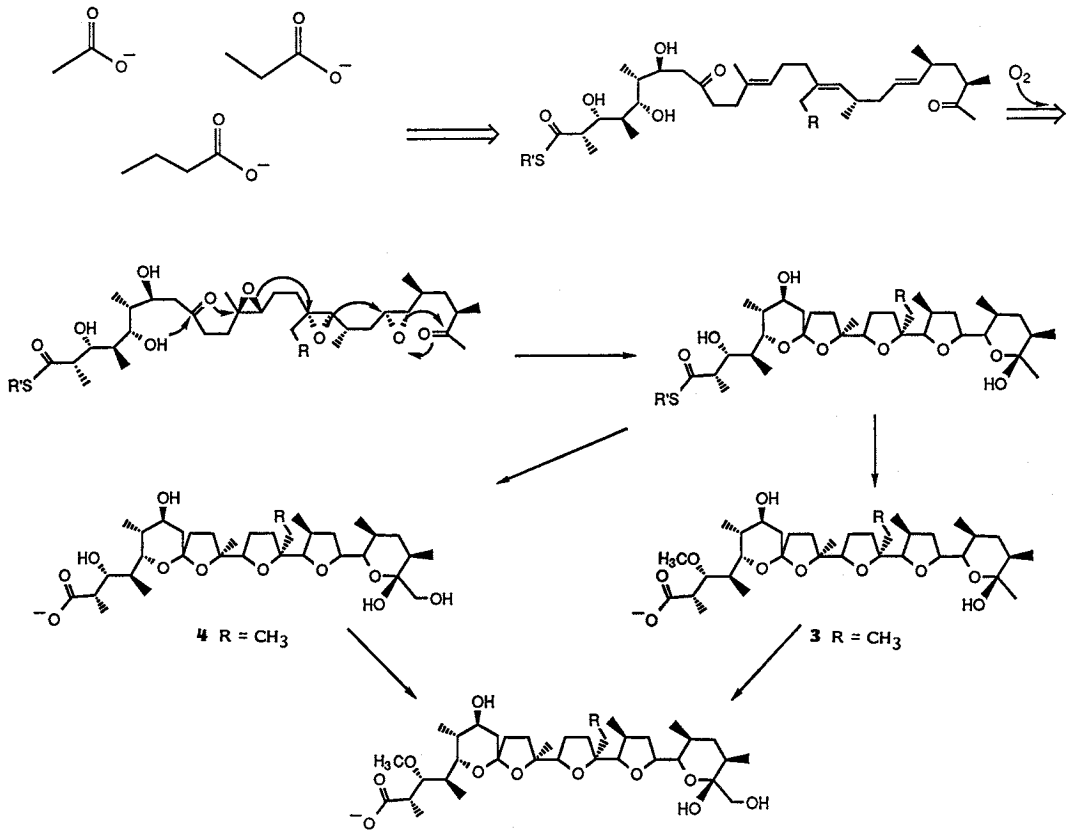
There have been no other reports, to date, on the isolation of mutants specifically blocked in the monensin biosynthetic pathway. Also, no *bona fide* biosynthetic intermediates have been isolated, although 3-*O*-demethylmonensins A (**4**) and B have been recovered[12] as minor components from the fermentation broth of a normal producing strain.

Materials and Methods

Media and Strains

The strain used in this work was *S. cinnamonensis* A3823.5, which was kindly donated by the

Scheme 1. The proposed biosynthetic pathway to monensins A and B.



Monensin A (1) R=CH_3
Monensin B (2) R=H

Eli Lilly and Company. This organism and all of the mutants were maintained on a solid medium which contained (per litre); Bacto-soytone (10 g), glucose (10 g), Difco-agar (20 g) and $CaCO_3$ (1 g), adjusted to pH 7 prior to sterilisation.

The minimal medium used is that described by HOPWOOD *et al.*[13], and comprised: Bacto-agar (10 g), L-asparagine (0.5 g), K_2HPO_4 (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), glucose (10 g), in distilled water (1,000 ml), pH 7.0~7.2.

The liquid medium-1 contained (per litre distilled H_2O); soybean flour (25 g), glucose (30 g), $CaCO_3$ (1 g), $MnCl_2 \cdot$hydrate (200 mg), $Fe_2(SO_4)_3 \cdot$hydrate (300 mg), KCl (100 mg), soybean oil (15 g), methyl oleate (20 g, technical grade) and lard oil (5 g). For screening mutants, this medium (7 ml) was dispensed in 50 ml plastic bottles, each closed with a foam bung, and sterilised prior to inoculation. For large scale cultures, a two stage fermentation was used; stage-1 (per litre distilled H_2O), glucose (5 g), soybean flour (15 g), potato dextrin (20 g), yeast extract (2.5 g) and $CaCO_3$ (1 g), dispensed as 60 ml in a wide neck Erlenmeyer flask (500 ml), and closed with a cotton wool bung before sterilisation. Growth was for 20 hours at 30°C on an orbital shaker; stage-2 used the same liquid medium-1 as above, dispensed as 60 ml amounts in a 500-ml wide neck Erlenmeyer flask, and following a 5% inoculum from stage-1, growth was at 30°C on an orbital shaker (300 rpm), for 5 days.

Mutagenesis

A *S. cinnamonensis* spore suspension (20 ml, *ca.* 10^8 spores) was irradiated batchwise with UV light so as to achieve a 99.0~99.8% kill rate. The survivors were spread onto the solid medium, and left

at 30°C for 5 days. From the well sporulated plates, a spore suspension was prepared, and used to inoculate new agar plates. From these plates well separated and sporulated colonies, whose growth characteristics appeared identical to the wild strain, were picked for further analysis. Individual colonies were transferred into liquid medium-1 (7 ml) and incubated at 30°C with shaking. The whole broth was then assayed for antibiotic production.

Assay

A TLC colorimetric assay was employed to detect monensin production in liquid cultures. Thus a sample of the whole fermentation broth was applied to a silica TLC plate, which was developed by elution with ethyl acetate. After drying, the plate was sprayed with a solution containing vanillin (3 g), MeOH (97 ml) and conc H_2SO_4 (2.4 ml), and dried by heating at 80°C. The polyether antibiotics generate a bright red spot. About 10 ng of monensin can be detected, on the TLC plate, in this way.

Selection of Mutants

In the first round of screening, 1,008 individual colonies were picked from agar plates and grown in liquid medium-1 (7 ml, each), as described above. After assaying each for monensin production, nine were found not to produce detectable levels of antibiotic, a further 20 produced monensin at very low levels (*ca.* 10~20-fold reduction over normal levels), and one, mutant DMA300, produced a compound that stained bright red-orange in the TLC-vanillin assay with an Rf 0.49, that was clearly different from monensin A (purple-red with Rf 0.39).

All of the non-producing mutants, and mutant DMA300, were tested for auxotrophy according to the method described by HOPWOOD *et al.*[18], by plating onto minimal medium containing the selected growth factors. None appeared to be auxotrophs for the common amino acids (in single letter code R, F, Y, W, M, C, T, I, V, L, S, G, H, E, P and K), nucleotide bases (U, T, A and G) and cofactors (nicotinamide, *p*-aminobenzoic acid, pyridoxin and riboflavin) tested.

Isolation of 26-Deoxymonensin A (3)

For the large scale isolation of the novel metabolite produced by mutant DMA300, a two stage fermentation was carried out. Thus the whole fermentation broth from four 60 ml cultures was centrifuged (10,000 rpm, 10 minutes, Beckman JA14 rotor), and the supernatant extracted with ethyl acetate (4×200 ml). The combined organic layers were dried (Na_2SO_4), and evaporated *in vacuo*, to give a brown oil (1.5 g). The pelleted cells were also extracted by stirring with ethyl acetate, filtering, and evaporation of the extract *in vacuo*, to again afford a brown oil (2.1 g). The extracts were combined at this stage.

A portion of the extract (2.0 g) was applied to a column of Sephadex LH-20 (75×2.5 cm), and eluted with dichloromethane. Fractions containing 26-deoxymonensin A were combined, evaporated, and the residue in diethyl ether was washed with satd aq $NaHCO_3$, dried over $MgSO_4$, and evaporated *in vacuo*. The resulting oil was chromatographed on a Florisil column eluting firstly with ethyl acetate and then with MeOH, and the desired fractions were evaporated, and the residue chromatographed again on Florisil eluting with ethyl acetate, to afford 26-deoxymonensin A (3) as an oil $[\alpha]_D^{20}$ +25.6° (*c* 0.45, CH_2Cl_2); *m/z* (electron impact (EI) mode) 676.4158 (calcd mass for $C_{36}H_{61}O_{10}Na$ 676.4162). See text for NMR data. Insufficient deoxymonensin B was obtained in pure form for full spectroscopic characterisation. The crude extract also contained small amounts of Na-monensin A (>10-fold lower amounts). The monensin A and deoxymonensins A and B were easily distinguished by TLC (silica plate, eluting with EtOAc) using the vanillin reagent:

Deoxymonensin A Na-salt; Rf 0.49; bright red with vanillin.

Deoxymonensin B Na-salt; Rf 0.41; bright red with vanillin.

Monensin A Na-salt; Rf 0.39; purple-red with vanillin.

Monensin B Na-salt; Rf 0.29; purple-red with vanillin.

Methyl Ester of 26-Deoxymonensin A

Na-26-deoxymonensin A (45 mg) in ethyl acetate (3 ml) was washed twice with dilute aqueous HCl (0.1 M, 2 ml). The organic phase was treated immediately with excess diazomethane in diethyl ether, and the solvents were removed *in vacuo*. The product was purified by TLC (silica gel, eluting ethyl

Table 1. Assignments of the ^1H NMR spectrum of 26-deoxymonensin A, recorded in $CDCl_3$, relative to TMS (δ 0.00).

Chemical shift	Assignment
4.45	5-H
4.39	20-H
4.06	17-H
3.96	21-H
3.89	7-H
3.56	13-H
3.39	3-H
3.36	OCH_3
2.58	2-H
2.32	19-H
2.31	18-H
2.28	4-H
2.26	15-H
2.21	6-H
2.05	10-H[a]
1.97	11-H[a]
1.90	8-H
1.78	14-H
1.73	10-H[a]
1.70	11-H[a]
1.68	30-H
1.68	32-H
1.65	8'-H
1.58	14-H
1.51	26-H
1.49	19-H
1.47	30-H
1.47	23-H
1.47	15-H
1.30	23-H
1.29	22-H
1.21	36-H
1.05	34-H
0.96	29-H
0.92	27-H
0.92	31-H
0.90	33-H
0.86	28-H

[a] The assignment of resonances for the two protons at C-10, and those for the two at C-11, cannot be made unambiguously. The other resonances are assigned from 2D NMR data.

Table 2. Chemical shifts of the ^{13}C (^1H) NMR spectrum of 26-deoxymonensin A, recorded in $CDCl_3$, relative to TMS (δ 0.00).

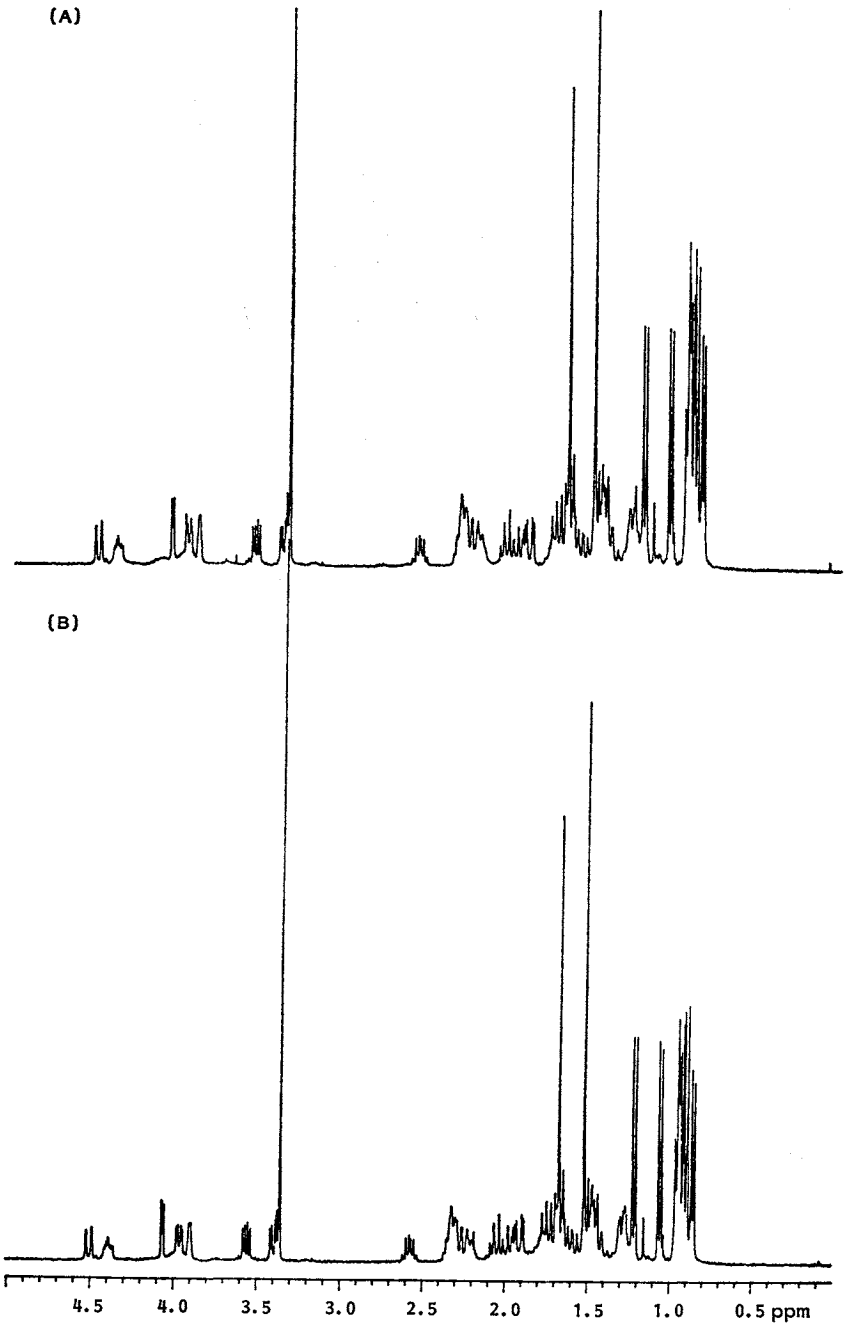
Chemical shift (multiplet structure)	Assignment
183.20 (s)	C-1
107.98 (s)	C-9
97.83 (s)	C-25
86.34 (s)	C-16
85.24 (s)	C-12
84.57 (d)	C-17
82.82 (d)	C-3
82.64 (d)	C-13
77.98 (d)	C-20
73.85 (d)	C-21
70.89 (d)	C-7
66.20 (d)	C-5
57.91 (d)	C-35
44.32 (d)	C-2
40.62 (d)	C-24
38.90 (t)	C-10
36.32 (t)	C-23
35.58 (d)	C-4
34.88 (d)	C-6
34.19 (d)	C-18
34.15 (t)	C-8
33.47 (t)	C-11
33.23 (d)	C-22
32.98 (t)	C-19
31.17 (t)	C-15
29.73 (t)	C-30
28.36 (q)	C-32
26.53 (t)	C-14
25.93 (q)	C-26
17.61 (q)	C-28
17.33 (q)	C-27
16.17 (q)	C-36
15.89 (q)	C-29
10.09 (q)	C-33
10.05 (q)	C-34

acetate - dichloromethane, 4:3) to afford an oil. *m/z* (EI mode) 668.4500 (M^+, 1%) (calcd mass for $C_{37}H_{64}O_{10}$ 668.4499), 651 ($M-H_2O$, 16%), 633 ($M-2H_2O$, 30%), 441 (74%), 423 (67%), 407 (50%), 405 (37%), 343 (100%), 195 (92%); *m/z* (fast atom bombardment (FAB) mode thioglycerol matrix) 691 (M^++Na).

Preparation of 26-Deoxymonensin A

Addition of Methyllithium to the C_{35}-Lactone **5**: Monensin A (**1**) Na-salt was converted to the known deoxymethyl lactone **5** by treatment with sodium *meta*-periodate, as previously described[2,4]. Methyllithium (1.3 M, 0.5 ml, 0.65 mmol) was added to a stirred solution of 105 mg (0.16 mmol) of **5** in 4 ml of anhydrous THF at −78°C under nitrogen. After 25 minutes the reaction was quenched by addition of saturated aq NH_4Cl. The mixture was allowed to warm to room temperature, diluted

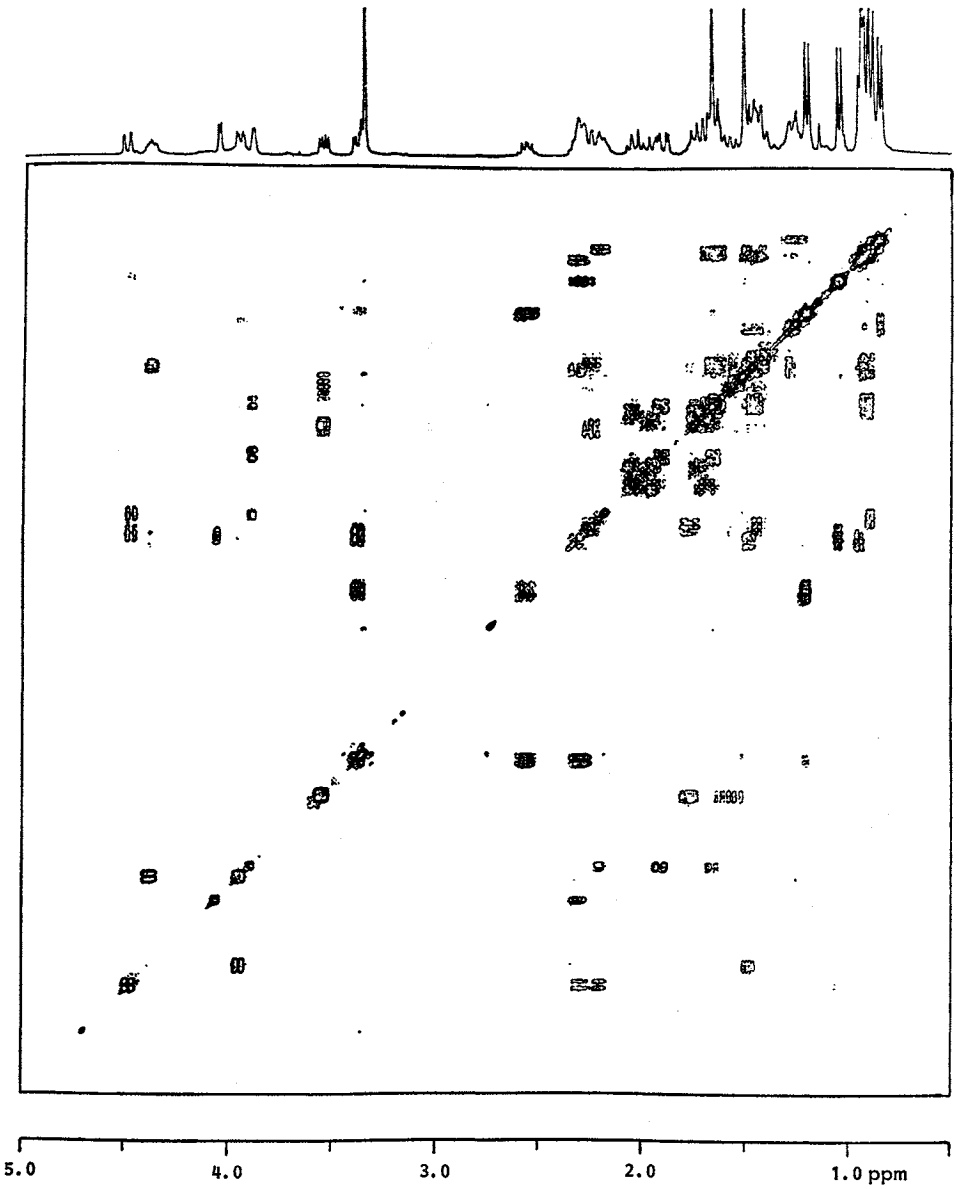
Fig. 1. 360 MHz ^1H NMR spectra of 26-deoxymonensin A.
(A) Isolated from *Streptomyces cinnamonensis* mutant DMA300.
(B) Prepared synthetically from monensin A.



The spectra were recorded in $CDCl_3$ as solvent, under identical spectrometer conditions. See Table 1 for assignments.

with water, and extracted with ether. The ether extract was washed with satd NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting oil was chromatographed on silica gel ($CHCl_3$ - MeOH, 100 : 2) to give 65 mg (60% yield) of 26-deoxymonensin A (**3**) Na-salt as an amorphous

Fig. 2. 2D ^{1}H-^{1}H COSY-90 spectrum of 26-deoxymonensin A (**3**), recorded in $CDCl_3$.



The spectrum was recorded and processed on a Bruker AM360 spectrometer, using the standard Bruker microprogram and software with the following parameters: F2 dimension 2K data points, F1 dimension 512W zero filled to 1K, spectral width in F2=1,623 Hz, 1 s relaxation delay, transformed with unshifted sine-bell squared function, and plotted after symmetrization.

solid. **3**-Na: MP 143~153°C (recrystallization from moist ether); IR $\lambda_{max}^{CHCl_3}$ cm^{-1} 3600~3000, 2960, 2920, 2870, 1575, 1460, 1105, 1095, 1074. The structure of **3** was confirmed by ^{1}H and ^{13}C NMR, ^{1}H-^{1}H homo correlation spectroscopy (COSY), ^{1}H-^{13}C hetero COSY, and $^{13}C(^{1}H)$-2D *J*-resolved spectroscopy (see Tables 1 and 2 and Figs. 1 and 2).

Photodeoxygenation of Monensin A 26-Monoacetate (**6**): Monensin A (500 mg, 0.75 mmol) was partially acetylated by reaction with 2 ml of acetic anhydride in 5 ml of pyridine for 23 hours at room temperature. After addition of ice-water and extraction with ether, the organic layer was

washed sucessively with 0.1 N NaOH, 1 N HCl, and satd NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. Silica gel chromatography ($CHCl_3$ - MeCN, 3 : 2) gave 154 mg of the derived 26-monoacetate **6** and 172 mg of the corresponding 7,26-diacetate[14]. ^1H NMR (**6**, $CDCl_3$) δ 0.85~0.98 (15H, 5×CH_3), 1.11 (3H, d, J=6.9 Hz, 34-CH_3), 1.25 (3H, d, J=6.5 Hz, 36-CH_3), 1.49 (3H, s, 32-CH_3), 2.09 (3H, s, CH_3CO), 2.65 (1H, m, 2-H), 3.21 (dd, J=2.3 and 9.8 Hz, 3-H), 3.36 (3H, s, CH_3O), 3.49 (dd, J=6.9 and 8.3 Hz, 13-H), 3.74 (1H, m, 7-H), 4.03 (d, J=4.1 Hz, 17-H), 4.0~4.1 (1H, m, 21-H), 4.1 (1H, m, 5-H), 4.17 (d, J=11.6 Hz, 26-H_a), 4.23 (d, J=11.6 Hz, 26-H_b), 4.3 (1H, m, 20-H). The monoacetate **6** (14.9 mg, 0.020 mmol) was dissolved in 12 ml of hexamethylphosphoramide - water (95 : 5) and irradiated in a quartz test tube for 4.5 hours with 254 nm UV light (General Electric Co., Type G25T8). After addition of water, the reaction mixture was extracted with ether, which was back-extracted with water, dried over Na_2SO_4, and concentrated under reduced pressure. Purification of the resultant oily residue by preparative TLC (silica gel, EtOAc - EtOH, 95 : 5) gave 1.3 mg (10%) of 26-deoxymonensin A (**3**).

Preparation of [24,26-$^2H_4$]- and [24,26-^{3}H]-26-Deoxymonensin A

A solution of 40.0 mg (0.059 mmol) of 26-deoxymonensin A (**3**) in 3 ml of dioxane was treated with 2 ml of 0.1 N NaOD in D_2O at room temperature for 30 minutes. After saturation with NaCl and dilution with satd NaCl, the mixture was extracted with ether. Drying (Na_2SO_4) and concentration of the ether extract followed by silica gel chromatography (CH_2Cl_2 - acetone, 2 : 1) led to recovery of 28 mg (69%) of [24,26-$^2H_4$]-26-deoxymonensin A. The sites of labelling were established by a combination of ^1H, ^2H and ^{13}C NMR analysis as described in the text. In like manner, [24,26-^{3}H]-26-deoxymonensin A was prepared from 11 mg of **3** in 30 μl of dioxane by treatment with 4 μl of 2 N NaOH and 18 μl of tritiated water (18 mCi/mmol). After preliminary chromatographic purification, the recovered tritiated product (6.6 mg) was diluted with 3.3 mg of unlabelled **3**, and recrystallized to constant activity (4.0×10^9 dpm/mmol) from Et_2O - MeOH - H_2O.

Feeding of [24,26-^{3}H]-26-Deoxymonensin A to *S. cinnamonensis*

A solution of 5.48 mg of [24,26-^{3}H]-26-deoxymonensin A in 4.0 ml of EtOH was dispensed in equal portions to twenty 50-ml cultures of *S. cinnamonensis* A3823.5 which had been grown for 72 hours as previously described[4]. After an additional 48 hours the resulting monensin was isolated and purified in the usual manner[4]. Recrystallization to constant activity from EtOAc gave 183 mg of monensin (9.9×10^3 dpm/mmol), corresponding to a specific incorporation of less than 0.00025% (total incorporation <0.02%).

Isolation of [*Methoxy*-^{14}C]-26-deoxymonensin A and Incorporation into Monensin A

A solution of [*methyl*-^{14}C]methionine (250 μCi, 50 mCi/mmol) and unlabelled methionine (20 mg) in distilled water (10 ml), was filter sterilised and added in three equal portions to a single shake flask culture (60 ml) of the mutant DMA300, 48, 58 and 72 hours after inoculation. After 6 days on a rotary shaker at 30°C the Na-26-deoxymonensin A (specific activity, 1.0×10^8 dpm/mmol) was isolated by the procedure described above. Subsequently, this material (14 mg) in EtOH (300 μl) was added in two equal portions to a single shake flask culture of *S. cinnamonensis* A3823.5, 48 and 72 hours after inoculation. After 6 days on a rotary shaker at 32°C monensin A Na-salt was isolated in the usual way, to afford material with a specific activity 5.1×10^4 dpm/mmol, corresponding to a specific incorporation of 0.0051%.

Results and Discussion

Preparation of Mutants

In the initial studies short wavelength UV light was chosen as a convenient mutagen for *S. cinnamonensis*. The assay used to detect non-producing organisms relies upon a chemical reaction between the antibiotic and an acidic solution of vanillin; the reaction generates a bright red material of unknown structure(s). This assay was chosen in preference to a bioassay against a standard or-

ganism such as *Bacillus subtilis*, since it was apparent that *S. cinnamonensis* produces other materials that also have a weak antimicrobial activity against this Gram-positive organism. On the other hand, this chemical assay, when used with TLC, is a highly specific and very sensitive method of detecting monensin production in liquid medium, the only disadvantage being that the screening procedure is labour intensive.

Following exposure to UV light, spores of *S. cinnamonensis* were first grown *en masse* on a non-selective agar medium, and a spore suspension was made from the resulting culture. Non-producing mutants were then sought amongst these expressed spores by picking individual colonies from a new agar medium into a single stage liquid fermentation broth. After growth on a rotary shaker the whole broth could be assayed for monensin production. Of the 1,008 individual colonies tested in this way 9 were found to produce undetectable levels of the antibiotic, and one other (mutant DMA300) was seen to produce a compound that by TLC migrated faster than monensins A and B, although it still reacted with vanillin to generate a bright red material, this being the typical response of a polyether antibiotic. These ten mutants were tested for auxotrophy by plating onto agar minimal medium containing the selected growth factors, which included, in separate experiments, the common amino acids (in single letter code, R, F, Y, W, M, C, T, I, V, L, S, G, H, E, P and K), nucleotide bases (U, T, A and G) and cofactors (nicotinamide, *p*-aminobenzoic acid, pyridoxin and riboflavin); none of the mutants were found to be auxotrophic for these materials.

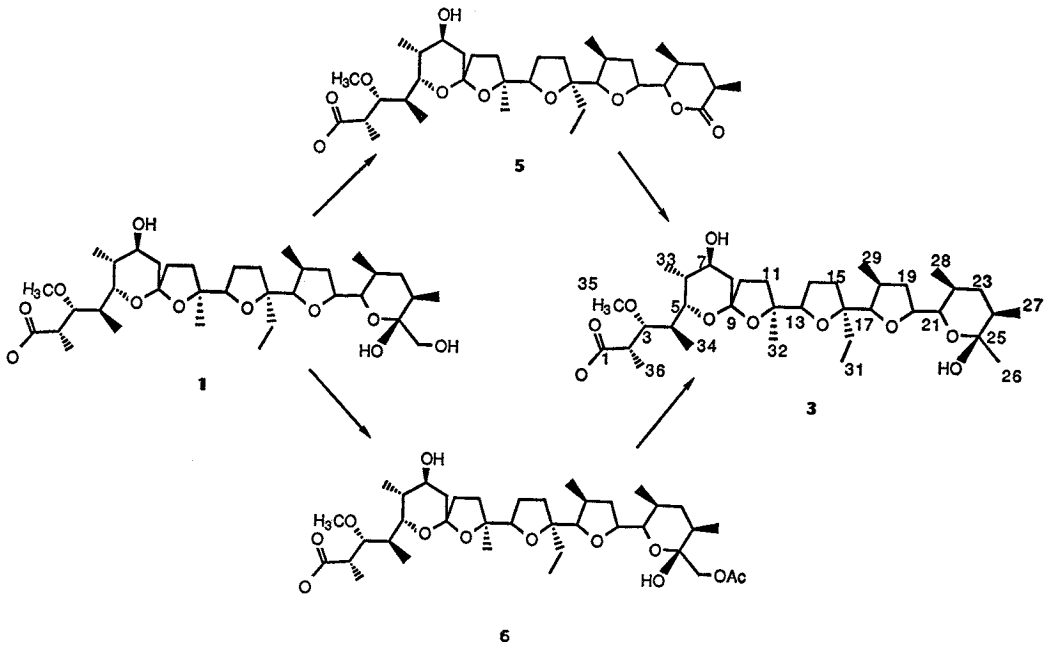
Extraction and Structure Determination

Since mutant DMA300 was producing a novel compound, either a biosynthetic intermediate or a shunt metabolite, this organism was selected for further study. After growth in liquid culture, the new metabolite could be purified following the protocol described in the Experimental section. Unlike monensins A and B, the material on silica gel was unstable during isolation, and chromatography led to substantial losses; this chromatography support was therefore avoided during the purification. In addition, chromatography on Sephadex LH-20 in methanol caused the partial conversion of the 26-deoxymonensin A into a derivative, tentatively identified as the corresponding 25-methoxyketal. However, eluting the crude extract through Sephadex LH-20 in methylene chloride gave fractions containing largely the required deoxymonensins A and B, in about a 10:1 ratio. The final purification steps involved rapid chromatography twice on Florosil, to afford the product as an oil. The deoxymonensin A was again unstable on this chromatographic support, and insufficient quantities of the 26-deoxymonensin B were obtained for a full spectrosocopic characterisation. Also present in some fractions was a polyether antibiotic (generates red pigment upon reaction with acidic vanillin), identified as monensin A Na-salt. The presence of the parent ionophore in the extract, albeit at a low level ($>$10-fold below the titre of 26-deoxymonensin A), indicates that the mutant is leaky.

The high resolution EI-MS of the new material showed a molecular ion consistent with the molecular formula $C_{36}H_{61}O_{10}Na$, indicating a nominal mass of 676, this being 16 mass units lower than monensin A sodium salt ($C_{36}H_{61}O_{11}Na$ 692). Also, exactly like the EI-MS of Na-monensin A, the base peak appears at (M$-$75), due to loss of (CO_2+OCH_3) (*m/z* 601). The sodium free form, after esterification with diazomethane, showed a molecular ion in the EI-MS at 668.4500 (calcd for $C_{37}H_{64}O_{10}$ 668.4499), and at *m/z* 691 $(M+Na)^+$ in the FAB-MS from a thioglycerol matrix, consistent with the expected structure for the methyl ester of 26-deoxymonensin A.

In all important respects, the mass spectra of the material extracted from *S. cinnamonensis*

Scheme 2. Preparative routes to 26-deoxymonensin A (3) from monensin A (1).

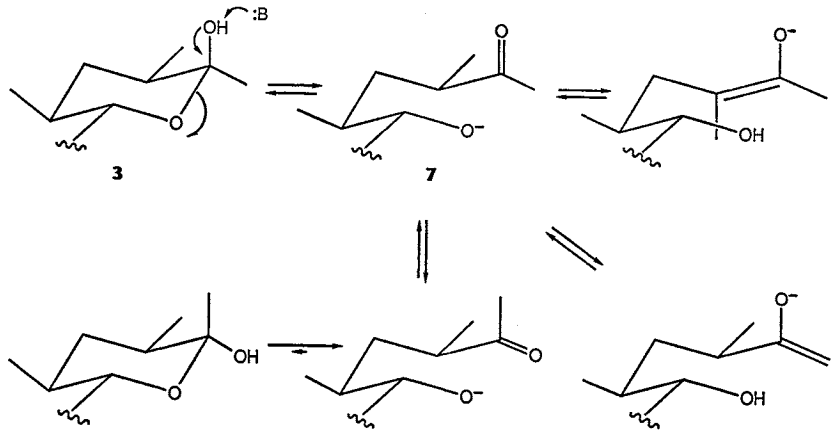


DMA300 and those from synthetically derived material, obtained as described below, were identical.

The major metabolite of *S. cinnamonensis* DMA300 was shown to be 26-deoxymonensin A Na-salt by ^{1}H and ^{13}C NMR spectroscopy. The 360 MHz ^{1}H and 2D COSY spectra of this metabolite are shown in Figs. 1A and 2. The assignments given in Table 1 followed straightforwardly from an analysis of these spectra, and were facilitated also through comparisons to the fully assigned ^{1}H spectrum available for Na-monensin A. The region between δ 4.5~3.0 is particularly informative since all of the protons attatched to oxygen-bearing carbons resonate here. Noticeable by their absence are resonances due to the two C-26 hydroxymethyl protons present in monensin A, whereas a new methyl resonance is present at δ 1.50, due to the methyl group now at position-26. The ^{13}C NMR assignments, given in Table 2, are consistent with this structure also; the signal at δ 64.9 in the spectrum of monensin A assigned to 26-CH_2 is absent, whereas a new methyl resonance is apparent at δ 26.0.

Unambiguous confirmation of the structure of 26-deoxymonensin A was obtained by direct comparison with an authentic sample of **3** prepared from monensin A. Thus reaction of methyllithium with the deoxymethyl lactone **5**, obtained by sodium periodate oxidation of monensin A, afforded the expected 26-deoxymonensin A (**3**) in 60% yield (Scheme 2). The assignment of the δ 1.50 singlet to the 26-CH_3 protons was confirmed by carrying out the same transformation using [$^{2}H_3$]methyllithium[15]. The ^{1}H NMR spectrum of the resulting [26-$^{2}H_3$]-26-deoxymonensin A was identical to that of unlabelled **3** except for the absence of the methyl singlet at δ 1.50. The configuration at C-25 of the hemiketal ring of **3** was shown to be identical to that of monensin A by conversion of monensin A (**1**) to the corresponding 26-monoacetate (**6**) which was photochemically reduced to **3** by irradiation at 254 nm in hexamethylphosphoric triamide - water[16,17]. Although the yield of the deoxygenated product obtained by the latter method was low (10%), the resulting sample of **3** was identical in all respects

Scheme 3. Base-catalysed exchange at C-24 and C-26 in **3**.



with that prepared by addition of methyllithium to the lactone **5**. These results also established that the conversion of **1** to **3** does not result in epimerization at any of the other stereogenic centres in the polyether. Semisynthetic 26-deoxymonensin A and **3** isolated from *S. cinnamonensis* DMA300 were found to be identical in all spectroscopic and physical properties. A comparison of the corresponding 360 MHz ^{1}H NMR spectra is given in Fig. 1.

Further support for the assignment of C-25 configuration in **3** came from exposure of 26-deoxymonensin A to mild aqueous base. Treatment of **3** with 0.1 N NaOD in dioxane for 30 minutes at room temperature resulted in surprisingly facile exchange of the C-24 and C-26 protons without any change in the relative configuration of either the 26-CH_3 or 27-CH_3 groups. In the ^{1}H NMR spectrum of the resulting labelled [24,26-$^2H_4$]-26-deoxymonensin A, the doublet corresponding to 27-H had collapsed to a singlet while the 26-H methyl singlet at δ 1.50 was no longer present. Consistent with the presence of deuterium at C-24 and C-26 was the observation of a deuterium-coupled triplet in the ^{13}C NMR spectrum at δ 40.53, corresponding to C-24 and the collapse of the C-26 methyl signal at δ 26.03, as well as the appearance of β-deuterium isotope shifts for C-27 (+0.11 ppm) and C-23. Moreover, the corresponding ^{2}H NMR spectrum showed a pair of signals at δ 1.35 and 1.50 due to the 24-D and 26-D, respectively. Exchange of either the 24-H or 26-H protons must occur through the corresponding ring-opened hydroxy ketone **7**. The remarkably mild conditions for base-catalysed exchange at the sites adjacent to the C-25 ketone are probably due to intramolecular participation of the neighboring C-21 hydroxyl group (Scheme 3). Under these conditions, the re-isolated hemiketal would be expected to be generated in the more stable configuration, with an axial C-25 hydroxyl group, as observed in monensin A.

Biosynthetic Intermediate or Shunt Metabolite?

In order to assess whether or not 26-deoxymonensin A (**3**) is an intermediate on the monensin biosynthetic pathway, a ^{14}C-labelled form was prepared by incorporating label from [*methyl*-^{14}C]methionine, in a shake flask feeding experiment with the mutant DMA300. The [*methoxy*-^{14}C]-26-deoxymonensin A was subsequently added to the parent strain, during the period of active monensin production. The monensin A recovered from this culture, however, showed only a very low incorporation of the ^{14}C-label (0.005% specific incorporation), most likely because the deoxymonensin A largely

failed to gain entry to the whole cells of the normal producing strain. In a complementary set of experiments, [24,26-^{3}H]-26-deoxymonensin A (4.0×10^9 dpm/mmol) was prepared by base catalysed exchange of **3** in the presence of tritiated water. Attempted incorporation of tritiated **3** into monensin A by administration to actively fermenting cultures of *S. cinnamonensis* again resulted in insignificant labelling of the derived **1**. These very low levels of observed incorporation, while inconclusive, are not sufficient to rule out the intermediacy of 26-deoxymonensin A in the biosynthesis of monensin A. In related work, we have also observed that labelled samples of the alternate penultimate metabolite, 3-*O*-demethylmonensin A (**4**), also fail to label monensin A when fed to cultures of *S. cinnamonensis*†. Whether 26-hydroxylation represents the final step in monensin biosynthesis is therefore still open to question and the precise roles of **3** and **4** remain unsettled††. It is conceivable that either hydroxylation or methylation normally occur at an early stage in the elaboration of the polyether and that **3** or **4** are in fact abortive shunt metabolites of the normal pathway. As a more likely possibility, it is intriguing to speculate that the producing organism may have developed an efficient mechanism for exporting and excluding polyethers from the interior of the cell, thereby accounting for the natural resistance to the effects of the ionophoric antibiotic. More conclusive answers to these questions must await the results of further investigation.

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† H. OIKAWA and D. E. CANE; unpublished work. Labelled 3-*O*-demethyl-26-deoxymonensin A, also was not incorporated into monensin.

†† Earlier studies have shown that **4** is a catabolite of monensin in both cattle and rats[18].

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