$NaHCO_3$ solution (2 × 60 mL). The $NaHCO_3$ extracts were neutralized with dilute HCl and extracted with ether (2 × 200 mL). The ethereal extracts were dried (Na_2SO_4) and evaporated to give a colorless residue (68 mg). Gas chromatography (column temperature, 190 °C; 15% SE-30 on Gas-Chrom Q; He flow rate, 55 mL min^{-1}) showed a major peak (t_R, 80 s) and a very small peak (t_R, 340 s). These were identified as benzoic acid and *p*-nitrophenol, respectively (see above). Subsequently, the residue was chromatographed on a column of silica gel (2.4 × 10 cm). Elution with benzene gave benzoic acid (0.049 g, 40%): mp 121–122 °C, identical with an authentic sample.

The original solution was evaporated on a steam bath and anisole removed under reduced pressure [53–55 °C (2 mm). GLC (conditions as above) of the residue showed five peaks with retention times of 90, 140, 340, 1200, and 1600 s, respectively. The components were collected and identified as *o*- and *p*-methoxyphenols, *p*-nitrophenol, *o*- and *p*-methoxyphenyl benzoates, respectively, by a comparison of their IR spectra with those of authentic samples.

Extraction with saturated $NaHCO_3$ solution removed *p*-nitrophenol (0.108 g, 78%): mp 113–114 °C. Extraction with 10% NaOH solution removed *o*- and *p*-methoxyphenols (0.052 g, 42%). The neutral residue (0.108 g, 48%) exhibited two peaks corresponding to *o*- and *p*-methoxyphenyl benzoates, the ratio of which was estimated quantitatively as ortho (16.3%) and para (31.7%) (diphenyl ether internal standard).

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Biosynthesis of the Boron-Containing Macrolide Antibiotic Aplasmomycin by *Streptomyces griseus*

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Abstract: The biosynthesis of aplasmomycin in *Streptomyces griseus* strain SS-20 was studied by feeding experiments with ^{13}C-labeled precursors. ^{13}C NMR analysis of the antibiotic samples biosynthesized from [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetate and from [$^{13}CH_3$]methionine showed that each half of the molecule is made up from seven intact acetate units providing carbons 1–14 and three methyl groups originating from methionine, which are located in positions 18, 19, and 20. Feeding experiments with [1,3-$^{13}C_2$]glycerol have shown unequivocally that glycerol is an intact precursor of the three-carbon starter unit of the polyketide chain. A possible mechanism for the conversion of glycerol into a suitable starter unit is proposed. Treatment of deboroaplasmomycin with boric acid led to reinsertion of the boron, demonstrating the chemical feasibility of a biosynthetic pathway involving first formation of the macrocyclic ring system followed by insertion of boron as the terminal step.

The boron-containing antibiotic aplasmomycin was isolated from a strain of *Streptomyces griseus*[1] obtained from shallow sea and mud as part of a screening program for new metabolites from marine microorganisms. Structure determination[2] by an X-ray analysis of the silver salt showed that it is a macrodiolide containing 40 carbon atoms and one boron. The compound is a symmetrical dimer which closely resembles boromycin,[3] the only other boron-containing natural product known. Its 16 chiral centers have identical configurations in the two halves of the molecule and its stereochemistry corresponds to that of boromycin at all centers except C-9 in one half of the boromycin molecule. In contrast to boromycin, aplasmomycin does not contain an amino acid moiety. Recently, two cometabolites of aplasmomycin, aplasmomycins B and C,[4] were isolated and identified as the monoacetate (at C-9) and the diacetate (at C-9 and C-9′) of aplasmomycin, respectively.

Aplasmomycin inhibits the growth of gram-positive bacteria, including mycobacteria, in vitro and is active against *Plasmodium berghei* in vivo.[1] Aplasmomycin and aplasmomycin B, but not aplasmomycin C and desboroaplasmomycin, show ionophoric properties, mediating net K^+ transport across a bulk phase.[4] The ion carrier activity correlates with antibiotic activity of these four compounds, aplasmomycin B having about equal antibiotic activity as aplasmomycin, whereas the other two are inactive. The metal ion specificity is rather pronounced for monovalent cations, with a preference for K^+, Rb^+, and Cs^+.

In the present communications we report results which establish the overall biosynthetic origin of aplasmomycin.

Results

The biosynthesis of aplasmomycin was studied in *Streptomyces griseus* strain SS-20 which was grown in a medium containing glucose and Kobu-cha (processed sea weed) and salt. On the basis of time-course studies, the labeled precursors were added to the cultures at 48 h after inoculation, and the cultivation was continued for an additional 48 h. Aplasmomycin could be isolated in yields of about 10 mg/L by chloroform extraction of the broth followed by preparative layer chromatography.

Because of the symmetry of the molecule, the ^{13}C NMR spectrum of aplasmomycin shows only 20 signals. An unequivocal assignment of every signal in the spectrum rests on the characteristic chemical shifts, multiplicities, single-frequency decoupling, comparison with several derivatives and model compounds, specific deuteration experiments, and analysis of one-bond carbon–carbon couplings of pairs of carbon atoms.[5]

The structure of aplasmomycin strongly suggests its formation by the polyketide (acetogenin) pathway. In analogy to the formation of most other macrolide antibiotics[6] one might expect aplasmomycin to be of a mixed acetate/propionate origin, i.e.,

(1) Okami, Y.; Okazaki, T.; Kitahara, T.; Umezawa, H. *J. Antibiot.* **1976**, *29*, 1019.
(2) Nakamura, H.; Iitaka, Y. Kitahara, T.; Okazak, T.; Okami, V. *J. Antibiot.* **1977**, *30*, 714.
(3) Dunitz, J. D.; Hawley, D. M.; Miklos, D.; White, D. N. J.; Berlin, Yu.; Marcesic, R.; Prelog, V. *Helv. Chim. Acta* **1971**, *54*, 1709.
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(5) Chen, T. S. S.; Chang, C.-j.; Floss, H. G. *J. Antibiot.* **1980**, *35*, 1316.
(6) (a) Corcoran, J. W.; Chick, M. In "Biosynthesis of Antibiotics"; Snell, J. F., Ed.; Academic Press: New York, 1966; pp 159–201; (b) Grisebach, H. "Biosynthetic Patterns in Microorganisms and Higher Plants"; Wiley: New York, 1967.

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Table I. Incorporation of Radioactively Labeled Precursors into Aplasmomycin

	precursor fed				aplasmomycin formed				
expt no.	compd fed	sp radioact, μCi/mmol	amount added, mmol/L	total radioact added, μCi/L	yield, mg/L	sp radioact of purified antibiotic, μCi/mmol	total radioact, μCi/L	sp incorp rate, %	tiritium retention, %
1	[1-^{14}C]acetate	4.9	4.88	24	10.7	7.5	0.11	152	
2	[2-^{14}C]acetate	1.6	5.38	10	10.6	3.6	0.05	223	
3	[1-^{14}C]propionate	11.8	1.49	18	7.7	0.35	0.003	2.9	
4	[2-^{14}C]propionate	5.9	3.05	19	9.6	4.1	0.050	71	
5	[3-^{14}C]propionate	12.2	1.59	19	7.8	9.5	0.086	78	
6	[1-^{14}C]succinate	2.8	3.25	9	7.3	0.12	0.001	4.3	
7	[1-^{14}C]pyruvate	4.6	2.30	10	7.7	0.09	0.00086	1.9	
8	[3-^{14}C]pyruvate	4.7	2.56	12	9.3	2.5	0.029	55.5	
9	[1-^{14}C]lactate	7.0	2.45	1.7	7.4	0.06	0.00055	0.82	
10	[3-^{14}C]lactate	5.4	3.09	1.7	11.4	1.0	0.015	18.3	
11	[1-^{14}C]glycerol	3.9	4.3	17	2.1	6.59	0.0175	170	
12	[2-^{14}C]glycerol	17.4	1.06	18	7.8	14.5	0.14	83	
13	[$^{14}CH_3$]methionine	9.4	2.3	21	8.2	13.9	0.143	148	
14	[2-^{14}C,2-^{3}H]acetate	^{14}C 3.6 ^{3}H 5.1	4.38	^{14}C 15.7 ^{3}H 22.3	14.0	^{14}C 17.4 ^{3}H 6.5	^{14}C 0.305 ^{3}H 0.12	^{14}C 484 ^{3}H 127	26
15	[1-^{14}C,1-^{3}H]glycerol	^{14}C 10 ^{3}H 11	1.63	^{14}C 16.5 ^{3}H 18.5	10.5	^{14}C 15.05 ^{3}H 4.86	^{14}C 0.197 ^{3}H 0.065	^{14}C 148 ^{3}H 44	30

Table II. ^{13}C Abundances and ^{13}C–^{13}C Couplings in Aplasmomycin Obtained from Feeding Experiments with ^{13}C-Labeled Precursors

		rel ^{13}C abundnace in aplasmomycin biosynthesized from:				
carbon no.	chemical shift, δ_C	[1-^{13}C]acetate	[2-^{13}C]acetate	[$^{13}CH_3$]-methionine	[1,3-$^{13}C_2$]-glycerol	$^{1}J_{C-C}$,[a] Hz
1	170.4	22.4	1.0	[b]	1.1	65.0
2	78.2	1.2	18.5		4.1	65.0
3	106.0	18.5	0.9		1.1	47.6
4	32.9	0.9	15.0		5.8	47.6
5	28.6	17.0	1.1		0.9	31.7
6	25.0	1.1	14.0		5.3	31.7
7	79.5	19.3	0.9		1.2	39.1
8	39.0	1.0	12.7		5.8	39.1
9	79.3	19.8	1.2		1.0	39.1
10	32.1	1.1	14.0		5.7	39.1
11	128.0	13.0	1.0		1.1	72.1
12	131.8	1.2	12.5		5.8	72.0
13	76.4	14.9	1.1		1.0	34.7
14	36.0	1.1	15.2		5.8	34.7
15	80.4	0.9	1.1		7.9	
16	78.2	1.2	1.0		1.1	
17	19.4	1.1	1.1		8.0	
18	16.5	1.1	1.1	56.6	4.0	
19	12.9	1.1	1.1	56.6	4.0	
20	21.6	1.1	1.1	56.6	4.0	

[a] In aplasmomycin from [1,2-$^{13}C_2$]acetate. [b] Not enriched.

each half of the molecule would be made up of five acetate (A) and three propionate (P) units in a sequence (Me)P-A-A-A-P-A-P-A(COOH), with an additional C-methylation at the central propionate unit to give the *gem*-dimethyl structure. As an alternative, the *gem*-dimethyl moiety might have resulted from the use of an isobutyryl unit in the form of 2,2-dimethylmalonyl-CoA, as chain extension unit. On the other hand, the methyl branches might arise entirely by C-methylation of acetate units, as has been observed in the biosynthesis of lankacidin.[7]

A series of feeding experiments with ^{14}C-labeled precursors to the aplasmomycin-producing strain SS-20 of *S. griseus* were carried out to distinguish among these possibilities. As shown in Table I, the specific incorporation values for acetate, methyl-labeled methionine, and propionate are remarkably high. These figures are often greater than 100%, reflecting the very low dilution encountered, the incorporation of multiple units of a precursor, and the fact that the molecule is a dimer. These high specific incorporation values made it easy to further explore the mode of incorporation of acetate and propionate into the antibiotic by feeding ^{13}C-labeled acetate and methionine. The purified aplasmomycin was analyzed by ^{13}C NMR spectroscopy. The results are summarized in Table II. The two experiments with [1-^{13}C]- and [2-^{13}C]acetate clearly indicate that in each chain carbon atoms–14 are acetate derived and show the alternating labeling pattern typical of polyketides. The experiment with [1,2-$^{13}C_2$]acetate demonstrates that the seven molecules of acetate making up each chain are incorporated as intact two-carbon units, since the signal for each acetate-derived carbon shows the doublet due to coupling to the other ^{13}C nucleus of the same precursor unit. Three methyl groups in each chain are derived from the methyl group of methionine, and these were identified as carbons 18, 19, and 20. Hence the methyl branches in the chain are derived by C-methylation rather than by incorporation of propionate units into the chain.

These results thus establish the origin of the molecule, except for three carbons in each half, carbons 15, 16, and 17, which presumably represent the starter units of the two polyketide chains. As shown in Table I, [2-^{14}C]- and [3-^{14}C]propionate gave good specific incorporations, 71% and 78%, respectively, into aplasmomycin. Surprisingly, however, an experiment with [1-^{13}C]-propionate gave aplasmomycin in which no individual carbon was specifically labeled, including C-15 which was expected to be enriched from this precursor. An experiment with [1-^{14}C]-

(7) Uramoto, M.; Otake, N.; Cary, L.; Tanabe, M. *J. Am. Chem. Soc.* **1978**, *100*, 3616.

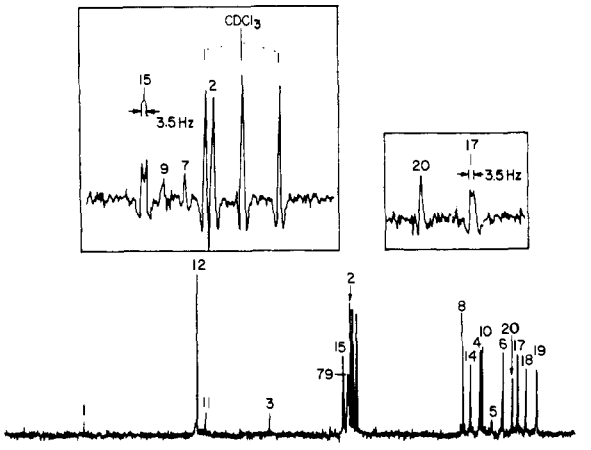


Figure 1. Carbon-13 NMR spectrum of aplasmomycin derived from [1,3-$^{13}C_2$]glycerol. Inserts: Scale expansions of the regions showing the C-15 and C-17 signals.

propionate (Table I) confirmed that propionate is not incorporated as an intact unit, but that following decarboxylation only carbons 2 and 3 are utilized indirectly. Kuhn–Roth oxidation of aplasmomycin derived from [2-^{14}C]- and [3-^{14}C]propionate gave acetic acid samples containing 13.3% and 13.8%, respectively, of the radioactivity of the antibiotic.[9] This suggests that C-2 and C-3 of propionate are converted into acetate via symmetrical intermediates, i.e., succinate and the Krebs cycle. The starter unit of the polyketide, thus, does not originate from propionate.

Several additional ^{14}C-labeled compounds were then tested in order to identify the precursor of the remaining three carbon starter units (Table I). Pyruvate, lactate, and succinate do not appear to be intact precursors of aplasmomycin. Although C-3 of both lactate and pyruvate is incorporated with reasonable efficiency, the carboxy carbon is not. Hence the situation is the same as with propionate, i.e., the compounds are only utilized indirectly following decarboxylation.

On the other hand, feeding experiments with [1,3-^{14}C]- and [2-^{14}C]glycerol gave remarkably high specific incorporations, ranging from 18% to 170%. Simultaneous feeding of an excess of cold acetate or methionine did not suppress the specific incorporation rate of glycerol. Kuhn–Roth oxidation of the aplasmomycin samples derived from [1,3-^{14}C]- and [2-^{14}C]glycerol gave sodium acetate containing 31% (of which greater than two-thirds was located in the methyl group) and 54% of the total radioactivity, respectively. Glycerol being a metabolically very active molecule, one can, of course, expect considerable indirect incorporation; however, the above data are suggestive of a more specific utilization. To unequivocally test this question, we synthesized and fed [1,3-$^{13}C_2$]glycerol and subjected the resulting aplasmomycin to ^{13}C NMR analysis. As shown in Table II, a considerable number of signals are enhanced, indicating enrichment in the following carbons: (1) the seven carbons C-2, C-4, C-6, C-8, C-10, C-12, and C-14, which are derived from C-2 of acetate, (2) the three carbons C-18, C-19, and C-20, which originate from methionine, and (3) the two carbons C-15 and C-17 of the presumed starter unit. The latter two signals show the highest enrichment, and the presence of a two-bond coupling with $^2J_{C_{15}-C_{17}}$ = 3.5Hz(Figure 1) indicates that these two carbons must

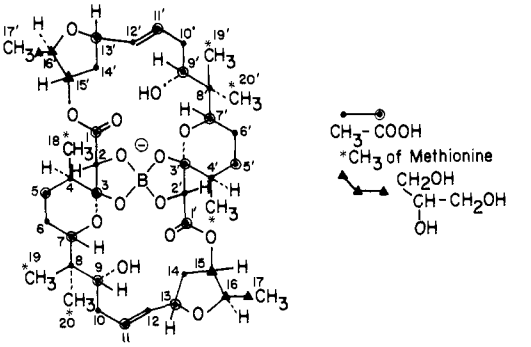


Figure 2. Biosynthetic origin of aplasmomycin.

originate from a single, intact glycerol unit. The three-carbon polyketide chain starter unit must therefore in some way be derived from an intact molecule of glycerol without any scission or rearrangement of the carbon skeleton.

A number of further experiments were carried out to set the stage for future studies on the mode of assembly of aplasmomycin from the established precursors. Since important mechanistic information can be deduced by following the fate of the various hydrogen atoms of the precursors during the biosynthesis, we carried out experiments with [2-^{14}C,2-^{3}H]acetate and [1,3-^{14}C,1,3-^{3}H]glycerol. The results (Table I, experiments 14 and 15) indicate in both cases a significant degree of tritium retention relative to ^{14}C. In the acetate case, the seven precursor molecules making up each half of aplasmomycin carry a total of 21 methyl hydrogens, of which at the most nine can appear in the product (one each at C-2, C-4, C-12, two each at C-6, C-10, and C-14). This corresponds to a maximum possible tritium retention (not considering isotope effects) of 43%. The observed value of 26% tritium retention, which corresponds to retention of five–six of the 21 hydrogens, indicates that nonspecific hydrogen exchange during the biosynthesis cannot be very extensive. The same is true with glycerol, although in this case it is not as easy to arrive at a value for the maximum possible tritium retention, because glycerol is converted to aplasmomycin by several different routes. The above experiments thus demonstrate the feasibility of determining the fate of these hydrogens by feeding deuterium-labeled precursors followed by NMR spectral analysis of the products. Finally, it was found that deboroaplasmomycin, which is available by treatment of aplasmomycin with 6 N HCl at room temperature,[4] upon shaking with boric acid solution is readily reconverted into the boron-containing form.

Discussion

The results presented here establish the basic building blocks of aplasmomycin as summarized in Figure 2. At least two unusual features characterize this biosynthesis. One is the origin of the methyl branches in the polyketide chain from the methyl group of methionine. This is in contrast to the biosynthesis of most macrolide antibiotics in which chain branches are formed by utilization of the appropriate homologues in place of acetate chain extension units, i.e., propionate units (in the form of methylmalonyl-CoA) for methyl branches[6,9] or butyrate units for two-carbon branches.[10] This mode of macrolide biosynthesis was first proposed by Woodward[11] and Gerzon et al.,[12] whereas the alternative C-methylation of an acetate-derived chain was suggested by Birch et al.[13] However, few examples of the operation of the latter route have so far been uncovered, namely, in addition to

(8) The theoretical values for intact incorporation of propionate into C-15 to C-17 would have been 100% for the acetic acid samples from both species of propionate. Indirect incorporation exclusively via acetate should have given 0% and 28.6% recovery of radioactivity in the acetic acids from [2-^{14}C]- and [3-^{14}C]propionate-derived aplasmomycin, respectively, if the conversion propionate → acetate involved straight oxidative decarboxylation and 14.3% in each acetic acid if the conversion proceeded via a symmetrical intermediate.

(9) This has been demonstrated, for example, in (a) erythromycin, ref 6; (b) methymycin; Birch, A. J.; Holzapfel, C. W.; Thompson, P. J.; Dutcher, J. D.; Djerassi, C. *Chem. Ind. (London)* **1960**, 1245; (c) picromycin, Omura, S.; Takeshima, H.; Nakagawa, A.; Miyazawa, J. *J. Antibiot.* **1976**, *29*, 316; (d) magnamycin A, Srinivasan, D.; Srinivasan, P. R. *Biochemistry* **1967**, *6*, 3111; (e) lucensomycin, Maezawa, I.; Rickards, R. W.; Gaudiano, G.; Nicolella, V. *J. Antibiot.* **1969**, *22*, 545.

(10) This has been demonstrated, for example, for (a) magnamycin, Omura, S.; Nakagawa, A. *J. Antibiot.* **1975**, *28*, 40; (b) Leucomycin, Omura, S.; Takeshima, H.; Lukacs, G. *Biochemistry*, **1977**, *16*, 2860; (c) tylosin, Omura, S.; Nakagawa, A.; Lukacs, G. *Tetrahedron Lett.* **1975**, 4503.

(11) Woodward, R. B. In "Festschrift Arthur Stoll"; Birkhauser Verlag: Basel, 1957; pp 524–544.

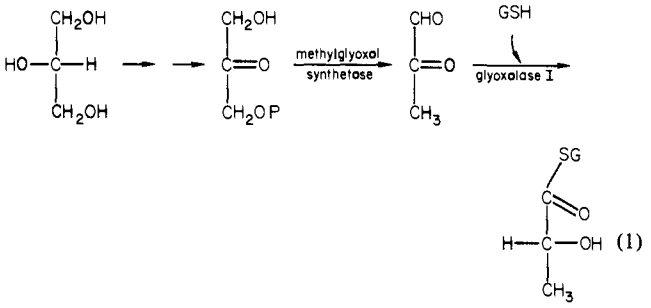
(12) Gerzon, K.; Flynn, E. G.; Sigal, M. V., Jr.; Wiley, P. F.; Monahan, R.; Quarck, C. U. *J. Am. Chem. Soc.* **1956**, *78*, 6396.

(13) Birch, A. J.; English, R. J.; Massy-Westropp, R. A.; Slaytor, M.; Smith, H. *J. Chem. Soc.* **1958**, 365.

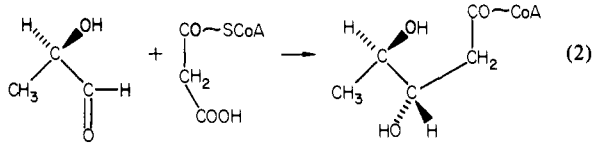
aplasmomycin and boromycin,[14] only the lankacidins.[7]

The second unusual feature of this biosynthesis is the mode of incorporation of glycerol and particularly the fact that it is a specific precursor of the three-carbon polyketide chain starter unit. Glycerol enters normal carbohydrate metabolism via *sn*-glycerol 1-phosphate and dihydroxyacetone phosphate. Further metabolism of [1,3-$^{13}C_2$]glycerol will then give [2-^{13}C]acetate via phosphoglyceraldehyde, phosphoglyceric acid, phosphoenolpyruvate, and pyruvate. This accounts for the labeling of aplasmomycin in carbons 2, 4, 6, 8, 10, 12, and 14. Phosphoglyceric acid can also be converted to serine, which from [1,3-$^{13}C_2$]glycerol would then be labeled in carbons 1 and 3. C-3 of serine can be transferred via the tetrahydrofolate system to homocysteine to give the methyl group of methionine; this accounts for the labeling of carbons 18, 19, and 20 of aplasmomycin. The transformation of glycerol into the three-carbon starter unit, however, is more difficult to rationalize, since pyruvate and lactate are apparently not intact precursors.

According to current biochemical knowledge, polyketide chains are initiated by thioesters of carboxylic acids as starter units. In the case of aplasmomycin one would predict a thioester of pyruvate or D-lactate as the most likely starter. Such a unit would have to be generated from glycerol without going through the corresponding free acid. As one possibility, this could be accomplished through the sequential action of two known enzymes, methylglyoxal synthetase[15] and glyoxalase I,[16] as shown in eq 1. The



resulting glutathione thioester of D-lactate could then serve directly as starter unit or could undergo thiol exchange with coenzyme A to give lactoyl-CoA. This pathway requires the assumption that added lactate or pyruvate cannot be activated by the organism. As an alternative, it would also be chemically feasible, although biochemically somewhat unprecedented, that an aldehyde serves as chain starter unit. Either methylglyoxal or D-lactic aldehyde formed from it by reduction could condense with malonyl-CoA to generate directly the alcohol oxidation stage found at C-15 in aplasmomycin (eq 2). Obviously, these schemes are at present entirely hypothetical and require further experimental exploration.



Little can be said at this point about the mode of assembly of the established precursor into the complete, boron-containing molecule. However, the observation that the boron can be easily reinserted into deboroaplasmomycin demonstrates the chemical feasibility of a biosynthetic route involving first the assembly of the entire macrolide structure followed by introduction of the boron as the last step as contrasted to a route on which the macrolide is built around the boron as a matrix.

Experimental Section

General Methods and Materials. ^{1}H NMR spectra were measured on a Varian EM-360 and a Varian FT-80 spectrometer. High-field ^{1}H NMR spectra were determined at 360 MHz on a Nicolet NTC-360 superconducting nuclear magnetic resonance spectrometer. ^{13}C NMR spectra were recorded on JEOL PFT-100, Varian FT-80, Nicolet NT-C-150, and Nicolet NTC-360 nuclear magnetic resonance spectrometers operating at 25.2, 20.16, 37.8, and 90.72 MHz, respectively. Chemical shifts are given in parts per million (ppm) relative to Me_4Si as internal standard or adjusted to Me_4Si by reference to the $CHCl_3$ resonance at δ_H 7.26 or δ_C 76.9. IR spectra were recorded on a Beckman IR-33 or a Beckman IR-3480 spectrometer. Mass spectra were determined on a Dupont 21-492 BR mass spectrometer, using either electron impact at 70 eV or chemical ionization with isobutane as reagent gas. Radioactivity measurements were done by liquid scintillation counting in a Beckman LS-250 or LS-7000 spectrometer using a commercial AQUASOL Universal SLC Cocktail (NEN) solution. Counting efficiencies were determined using a [^{3}H]toluene or [^{14}C]toluene internal standard. Preparative TLC was performed on 0.25 mm, 20 × 20 cm silica gel 60 F-254 plates supplied by Brinkmann. The solvent was benzene/ethyl acetate (50:50). Aplasmomycin was located by spraying with water. Radiolabeled compounds were purchased from Amersham-Searle or New England Nuclear and used without further purification. ^{13}C-Labeled compounds were obtained from Merck Sharp and Dohme. *S. griseus* SS-20 was kindly provided by Professor Y. Okami, Institute of Microbiol Chemistry, Tokyo. Kobu-cha seaweed powder was purchased from Fuji Shokuhin Co., Osaka, Japan. Synthetic sea salt was obtained from Aquarium Systems Inc., Lafayette, IN.

Culture Conditions. *S. griseus* SS-20 was maintained on agar slants (1.0% soluble starch, 0.1% casein, 1.7% agar, 500 mL of artificial sea water, 500 mL of distilled water, pH 7.4, before sterilization) grown at 27 °C for 2–3 weeks and stored at 4 °C. Spores of strain SS-20 cultivated on an agar slant were inoculated into 100 mL of Kobu-cha medium (1.0 g of Kobu-cha, 1.0 g of glucose, 3.0 g of NaCl, 100 mL of distilled water, pH 7.8) in a 500-mL Erlenmeyer flask and shaken at 27~28 °C on a New Brunswick rotary shaker at 300 rpm for 48 h. Four milliliters of this culture was transferred to 100 mL of the same medium in a 500-mL flask and shake-cultured for 4 days.

Isolation of Aplasmomycin. The culture broth was acidified to pH 6.5 with 1 N HCl and filtered through filter paper to remove the mycelia and the solid components of the broth. The filtrate was extracted 3 times with chloroform. The combined chloroform extract was dried over sodium sulfate and concentrated on a rotary evaporator to give a yellowish syrup of crude aplasmomycin. The residue was purified by preparative thin-layer chromatography.

[1,3-$^{13}C_2$]Glycerol. This was synthesized from diethyl [1,3-$^{13}C_2$]-malonate by oxidation with lead tetraacetate followed by reduction with lithium aluminum hydride. The overall yield was 72%. ^{1}H NMR (D_2O) δ 3.4 (dm, J = 148 Hz), 3.4 (m, H-2); CIMS, m/e (rel intensity) 95 (M^+ + 3, 73), 94 (M^+ + 2, 13.5), 93 (M^+ + 1, 13.5).

Deboroaplasmomycin. This was obtained by acid hydrolysis of aplasmomycin.[4] IR ($CDCl_3$) 3440, 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.80 (td), 5.48 (dd), 4.96 (dd), 4.75 (bd), 4.51 (dq), 4.42 (s), 3.82 (d), 3.54 (dd), 2.45 (ddd), 2.05 (m), 1.96 (d), 1.60 (m), 1.29 (d), 0.98 (d), 0.78 (s), 0.70 (s); ^{13}C NMR ($CDCl_3$) δ 14.9, 17.4, 20.6, 22.2, 26.1, 28.7, 33.7, 34.3, 38.5, 40.6, 75.4, 78.2, 79.4, 80.6, 80.9, 82.7, 100.5, 128.7, 133.1, 169.0.

Conversion of Deboroaplasmomycin to Aplasmomycin. Deboroaplasmomycin (1 mg) was dissolved in 0.5 mL of dioxane. Two drops of boric acid solution (0.3 M, pH 6.5 or 8.3) was added and the reaction was allowed to proceed at room temperature. After 1 h the reaction was checked by TLC in ethyl acetate/benzene (50:50) and chloroform/methanol (97:3). A compound which was chromatographically identical with aplasmomycin was found. The solution was evaporated to dryness. The mass spectrum shows an ion at *m/e* 798 corresponding to the molecular ion for aplasmomycin.

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