with brine (2 × 25 mL), dried over $MgSO_4$, and filtered, and the volatiles were removed under reduced pressure. The residual oil was distilled bulb-to-bulb to give the ketones (≥95% GC). Further purification by preparatory GC gave the analytically pure ketones.

***trans*-Methylpropyl Phenyl Ketone.** For spectral data of this compound, see under the section General Procedure for the Preparation of Ketones.

[*R*]-(−)-2,2,4-Trimethylhexen-3-one: prepared from ([*R*]-1-methylpropyl)(*tert*-butyl)(3-(trimethylsiloxy)propoxy)borane with the reaction mixture heated to reflux 12 h, cooled, and worked up as described above; IR ν_{max} 1702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (t, J = 7 Hz, 3 H), 1.03 (d, J = 7 Hz, 3 H), 1.15 (s, 9 H), 1.33 (m, 1 H), 1.62 (m, 1 H), 2.91 (sext, J = 7 Hz, 1 H); MS, m/e (chemical ionization) 143 (100, M^+ + 1); MS, m/e (electron impact) 142 (1, M^+). Anal. Calcd for $C_9H_{18}O$: C, 76.06; H, 12.68. Found: C, 75.98; H, 12.86.

Epimerization of Cyclic Ketones. The ketone (0.1 mmol) was dissolved in 3 N sodium methoxide in methanol (0.5 mL) and stirred for 24 h at room temperature. The reaction was diluted with water (2 mL) and extracted with ether (1 mL). The organic phase was dried over $MgSO_4$ and filtered. An aliquot was analyzed by capillary GC (methyl silicone, 50 M).

Preparation of Ketals. The ketone (0.1 mmol) was dissolved in dichloromethane (0.5 mL) and cooled to −10 °C. [2*R*,3*R*]-(−)-2,3-butanediol (0.3 mmol) and chlorotrimethylsilane (0.6 mmol) were added sequentially. The reaction was stirred at −10 °C for 30 min, poured into saturated sodium bicarbonate solution, and extracted with ether (1 mL). The ethereal solution was dried over $MgSO_4$ and filtered. An aliquot was injected into capillary GC for analysis.

Further Studies on the Biosynthesis of the Boron-Containing Antibiotic Aplasmomycin

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Abstract: In the biosynthesis of aplasmomycin by *Streptomyces griseus*, seven atoms of deuterium from C-2 of acetate are incorporated per chain (one each at C-2, -10, and -12 and two each at C-6 and -14) and 4 atoms of oxygen from C-1 of acetate (one each at C-1, -7, -9, and -13). The two hydrogens of the *pro-R*-hydroxymethyl group of glycerol are incorporated into C-17, giving rise to a chiral methyl group of *S* configuration when (1*R*,2*R*)-[1-$^2H_1$,^3H]glycerol is used as substrate. The three methionine-derived *C*-methyl groups per chain are transferred stereospecifically with inversion of configuration, but racemization is observed during the formation of the methionine methyl group from stereospecifically labeled [3-$^2H_1$,^3H]serine. The stereochemical and precursor feeding experiments point to phosphoglyceric acid or phosphoenolpyruvate as the glycerol-derived polyketide chain starter unit, ruling out serine, methylglyoxal, and pyruvate and compounds derived from these. Mechanistic aspects of the modification of the initial polyketide chain are discussed.

Aplasmomycin (**1**) is a novel ionophoric macrolide antibiotic that was isolated from strain SS-20 of *Streptomyces griseus* obtained from a sample of sea mud.[1] Its structure, as determined by single-crystal X-ray analysis[2] and confirmed by synthesis,[3] is that of a symmetrical dimer built around a boron atom. It is closely related to boromycin, the first boron-containing antibiotic found in nature.[4] The two compounds have very similar conformations and identical configurations at all the asymmetric centers except C-9, but in contrast to boromycin, aplasmomycin does not contain the D-valine moiety. Two minor components of the fermentation, asplasmomycin B and C, have been isolated and identified as the monoacetate at C-9 and the diacetate at C-9 and C-9′.[5]

Studies on the biosynthesis of **1** have established the origin of its carbon skeleton as summarized in Scheme I.[6] Each half of the molecule represents a polyketide chain made up of a starter unit and seven acetate/malonate extension units. The latter are modified by C-methylation through transfer of three methyl groups 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

Scheme I. Structure and Biosynthetic Origin of Aplasmomycin

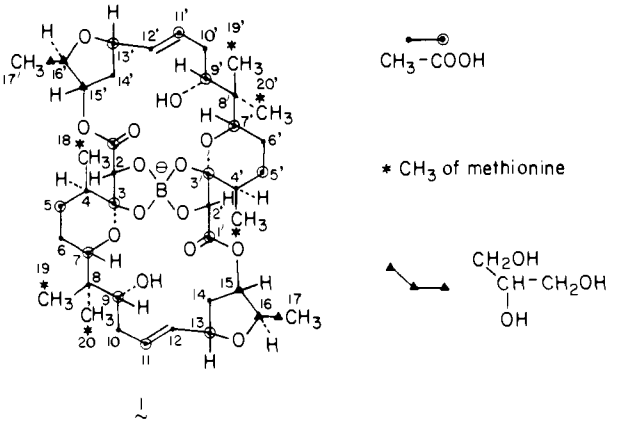

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Table I. Carbon-13 Levels and Fraction of ^{13}C Bonded to ^{2}H in Aplasmomycin Obtained from a Feeding Experiment with [2-^{13}C,2-$^{2}H_3$]Acetate

carbon no.	δ_c (ppm)	^{13}C enrichment (% above nat. abund)	$\Delta\delta_c$ (ppm)	fraction of ^{13}C bonded to $^{2}H^b$
2	78.2	19	0.28	C^2H: 10 ± 3%
4	32.9	19	no isotopically shifted peak[a]	
6	25.0	23	0.35	C^1H^2H: 11 ± 3%
			0.72	C^2H_2: 9 ± 3%
8	39.0	18	no isotopically shifted peak[a]	
10	32.1	21	0.30	C^2H: 12 ± 3%
12	131.8	18	0.39	C^2H: 10 ± 2%
14	36.0	23	0.29	C^1H^2H: 12 ± 3%
			0.57	C^2H_2: 6 ± 3%

[a] $^{13}C^2H$: <3%. [b] Comparative error ±2%; absolute error ±6% due to large error in areas of natural abundance peaks (av. 20 ± 6%).

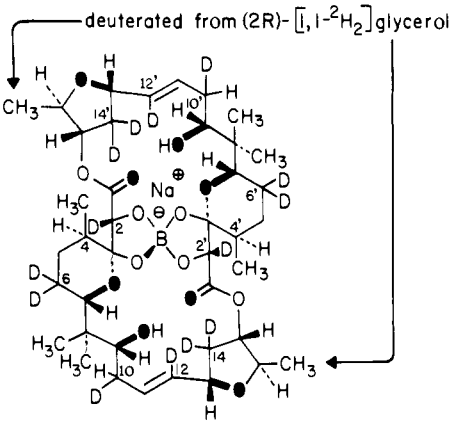
CoA, for methyl branches[7] or butyrate units for two carbon branches.[9] This mode of macrolide biosynthesis was first proposed by Woodward[10] and Gerzon et al.,[11] whereas the alternative C-methylation of an acetate-derived chain was suggested by Birch et al.[12] However, few examples of the operation of the latter route have so far been uncovered—in addition to **1** mainly boromycin[13] and lankacidin.[14] Also unusual is the origin of the starter unit which contrary to reasonable expectation is not formed from propionate, lactate, or pyruvate; rather, it was found that the three carbon atoms of the starter unit are derived intact from the three carbon atoms of glycerol.[6]

In this paper we report additional results that shed light on the nature of the starter unit and on mechanistic aspects of the biosynthesis of **1**.

Results

Incorporation of Acetate Hydrogen and Oxygen Atoms. In order to obtain further information on the mode of assembly and modification of the polyketide chain of **1** we determined the fate of the methyl hydrogen and carboxyl oxygen atoms of acetate during the biosynthesis. This was done with use of the isotope shift methodology[15,16] and precursors double labeled with ^{13}C and deuterium or ^{18}O. A sample of aplasmomycin (7 mg) biosynthesized from sodium [2-^{13}C,$^{2}H_3$]acetate (187 mg added to five 100-mL cultures) was analyzed by ^{13}C NMR spectroscopy with simultaneous ^{1}H and ^{2}H broadband decoupling. Several of the ^{13}C signals showed shoulder peaks toward higher field due to ^{2}H-induced isotope shifts. Enrichments and fractions of ^{13}C bonded to ^{2}H were measured from integrals of a spectrum obtained with the relaxation reagent, $Cr(acac)_3$, added to the sample and gated decoupling to provide partial suppression of residual nuclear Overhauser enhancement. The results are summarized in Table I. As expected, the resonances of C-2 and C-12 contain only a single isotopically shifted signal corresponding to the species $^{13}C^2H$, and the resonances of C-6 and C-14 display two isotopically shifted components corresponding to the species $^{13}C^1H^2H$ and $^{13}C^2H_2$. That the signal for C-4 does not show any ^{2}H-induced isotope satellite is especially interesting. Obviously, one of the original methylene hydrogens at this position is replaced by the methyl group. The other is apparently removed during the elimination of the oxygen function at C-5, which must proceed via a 4,5-ene rather than a 5,6-ene intermediate. The resonance of C-10 displays only one isotopically shifted component corresponding to the species $^{13}C^1H^2H$, in addition to the $^{13}C^1H_2$ resonance, indicating the presence of only one atom of deuterium at C-10. This suggests initial formation of a $\Delta^{10,11}$ double bond in the removal of oxygen from C-11 followed by double bond migration. Another possibility, initial formation of a 10,12-diene and furan ring closure by a 1,4-addition to this diene, is ruled out by data presented below. The deuterium distribution in **1** formed from [2-^{13}C,2-$^{2}H_3$]acetate is shown in Scheme II.

Scheme II. Distribution of Deuterium and ^{18}O in Aplasmomycin Biosynthesized from Acetate and Glycerol



D = deuterium from [2-^{13}C, $^{2}H_3$]acetate.
● = ^{18}O from [1-^{13}C, $^{18}O_2$]acetate. Heavy lines connect the ^{18}O to the carbon from which it originated.

Table II. Carbon-13 Enriched Carbons and Fraction of ^{13}C Bonded to ^{18}O in Aplasmomycin Obtained in a Feeding Experiment with [1-^{13}C,$^{18}O_2$]Acetate

carbon no.	δ_c (ppm)	$\Delta\delta_c$ (ppm)	fraction of ^{13}C bonded to ^{18}O
1	170.57	0.037	44%
3	106.18	no isotopically shifted peak	
5	28.77	no isotopically shifted peak	
7	79.73	0.031	46%
9	79.27	0.024	45%
11	128.17	no isotopically shifted peak	
13	76.88	0.031	43%

In an analogous experiment, sodium [1-^{13}C,$^{18}O_2$]acetate (444 mg, 63% ^{18}O, diluted with 1.78 g of nonlabeled material) was fed to 24 cultures and the resulting purified **1** (7 mg) was examined by ^{13}C NMR for the distribution of ^{18}O as evidenced by ^{18}O-shifted satellites of the ^{13}C signals. The results are summarized in Table II. The presence of one atom of ^{18}O each at C-1, C-7, and C-9 is seen, as expected. Unexpected is the absence of an ^{18}O satellite from the C-3 signal, indicating that the second oxygen at this position may not come from the acetate carboxyl group. A possible source of this oxygen might be the borate anion. Particularly interesting is the appearance of an ^{18}O satellite at C-13. This shows unambiguously that the oxygen of the tetrahydrofuran ring comes from the carbonyl group at C-13 rather than from C-16 of the starter unit. The ^{18}O distribution deduced in this experiment is summarized in Scheme II.

Nature of the Starter Unit. The finding that the polyketide starter unit is derived from glycerol rather than propionate and that neither [1-^{14}C]pyruvate nor [1-^{14}C]lactate are significantly incorporated led us to speculate that this unit might be derived

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Table III. Carbon-13 Distribution in Aplasmomycin Biosynthesized from D,L-[3-^{13}C]Serine

carbon no.	δ_c (ppm)	rel. ^{13}C abundance[a]
1	170.4[b]	1.4
2	78.2	2.4
3	106.0	1.4
4	32.9	2.2
5	28.6	1.1
6	25.0	2.3
7	79.5	1.1
8	39.0	2.1
9	79.3	1.0
10	32.1	2.7
11	128.0	1.3
12	131.8	2.9
13	not resolved from $CDCl_3$ signals	
14	36.0	2.8
15	80.4	1.1
16	78.2	1.2
17	19.4	1.4
18	16.5	8.8
19	12.9	8.7
20	21.6	8.7

[a] Relative to the abundance of C-9 = 1.0. [b] Assignments based on ref 18.

from dihydroxyacetone phosphate, an early product of glycerol assimilation, via the methylglyoxal synthase reaction. Thus, methylglyoxal and/or lactic aldehyde could be intermediates in the formation of the starter unit from acetate. Both compounds were synthesized, the latter as the racemate, carrying ^{13}C or ^{14}C in the methyl group. Feeding experiments showed specific incorporations of ^{14}C of 5–15%, but the NMR analysis of the products from the feeding of the ^{13}C-labeled precursors revealed only nonspecific labeling, mainly in the positions originating from C-2 of acetate, but no specific enrichment in carbon atoms 15–17 (data not shown). It seems likely that both compounds were, at least to some extent, metabolized to pyruvate and lactate, respectively, which then give rise to [2-^{13}C]acetyl-CoA.

Next we examined the possibility that serine might be the starter unit or a more immediate precursor of the starter unit. Glycerol can be converted to serine rather directly via dihydroxyacetone phosphate, phosphoglyceric acid, and phosphohydroxypyruvate. A feeding experiment with D,L-[3-^{13}C]serine (104 mg, 90% ^{13}C) to twenty 100-mL cultures gave 12.2 mg of purified **1** for NMR analysis. The data, summarized in Table III, show that the three methyl carbon atoms per chain derived from methionine are heavily enriched. This results from the transfer of C-3 of serine by serine hydroxymethyltransferase to the tetrahydrofolate system, reduction of 5,10-CH_2-H_4folate to 5-CH_3-H_4folate, and transfer of the methyl group to homocysteine by methionine synthase.[17] To a lesser extent one sees enrichment of the carbon atoms derived from C-2 of acetate, indicating that [3-^{13}C]serine is metabolized to pyruvate which is oxidatively decarboxylated to [2-^{13}C]-acetyl-CoA. However, there was no significant incorporation of ^{13}C from serine into carbon atoms 15–17. This rules out serine as a precursor of the starter unit and, because of the observed metabolism of serine to acetate, also argues against pyruvate as the polyketide chain starter.

To define the orientation of the glycerol molecule in the starter moiety, i.e., whether C-1 gives rise to C-17 and C-3 to C-15 or vice versa, and to obtain further information on the mode of the transformation we fed several deuteriated glycerol samples. [1,3-^{14}C]Glycerol was fed simultaneously with the samples to obtain a measure for the degree of incorporation of the carbon backbone. From earlier experiments[6] we know that about 25% of the label from [1,3-^{13}C]glycerol resides in the starter unit, about 60% in the carbon atoms derived from C-2 of acetate, and about

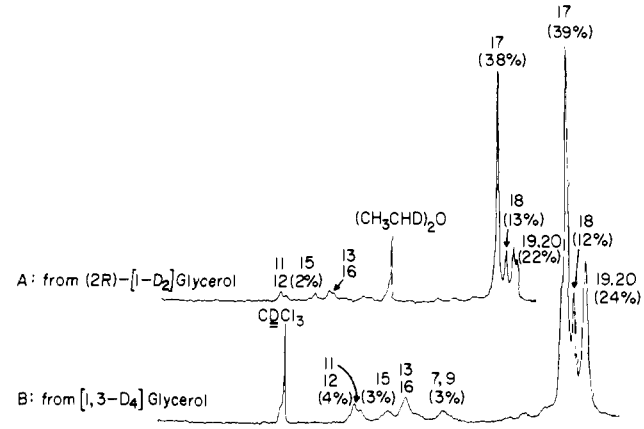

Figure 1. ^{2}H NMR spectra of aplasmomycin biosynthesized from deuteriated glycerol: (A) measured in $(CH_3CH_2)_2O$, (B) measured in $CHCl_3$. The percentage figures under the numbers of the carbon atoms refer to the fraction (relative percentage) of the total deuterium in the compound (about 16–26% absolute ^{2}H enrichment) residing at that carbon atom and its counterpart in the other chain. Signal assignments are based on earlier work.[18]

15% in the methionine-derived methyl groups. An experiment with [1,3-^{14}C,2-$^2H_1$]glycerol (312 mg, > 95% ^{2}H) gave purified aplasmomycin (13.4 mg from 20 flasks) with a specific incorporation of ^{14}C of 38%, but the mass spectrum indicated no incorporation of deuterium into the molecule. If deuterium had been retained in the formation of the starter unit, a deuterium enrichment of close to 10% would have been expected. This finding is consistent with conversion of glycerol into the starter unit via dihydroxyacetone phosphate.

(2*R*)-[1-$^2H_2$]Glycerol (244 mg, 98% ^{2}H) gave 16.5 mg of aplasmomycin from 19 flasks with a specific incorporation of the carbon backbone of 55%. On the basis of an earlier experiment with [1,3-^{14}C,^{3}H]glycerol,[6] which showed 30% tritium retention relative to ^{14}C, one would expect about 16% deuterium enrichment in this sample. The ^{2}H NMR spectrum (Figure 1, tracing A) shows that over one-third of the deuterium in each chain is located at C-17, the methyl group of the starter unit, with only 2% or less appearing at C-15. Hence, the *pro-R*-hydroxymethyl group of glycerol, the position which is phosphorylated by glycerol kinase, gives rise to C-17. As expected, some deuterium is incorporated into the methionine-derived methyl groups, indicating again appreciable metabolism of glycerol via serine to methionine. Very little of the deuterium appears in the positions derived from the methyl group of acetate. An experiment with [1,3-$^2H_4$]glycerol (314 mg, 98% ^{2}H) gave **1** (11.6 mg from 19 flasks) with 89% specific incorporation of the carbon backbone, which showed an almost identical ^{2}H NMR spectrum with that of **1** from (2*R*)-[1-$^2H_2$]glycerol (Figure 1, tracing B). In particular, there is no significantly greater incorporation of deuterium into the C-15 and C-16 methine groups. This is consistent with incorporation of glycerol via a starter unit containing C-15 (derived from C-3 of glycerol) at the oxidation state of a carboxyl group, and it rules out any migration of hydrogen from C-3 of glycerol to the neighboring carbon as would, for example, be encountered in the dismutation of methylglyoxal to a lactoyl-thioester by glyoxylase I.[19-21]

Stereochemistry of Methyl Group Formation. Another approach to defining the nature of the starter unit consists of establishing the stereochemistry of formation of the C-17 methyl group from C-1 of glycerol. It might be possible to rule out or to support certain pathways depending on whether or not their known stereochemistry agrees with that observed for formation of the 17-methyl group of **1** from a sample of glycerol chirally labeled at C-1. For example, if the starter unit were related to pyruvate,

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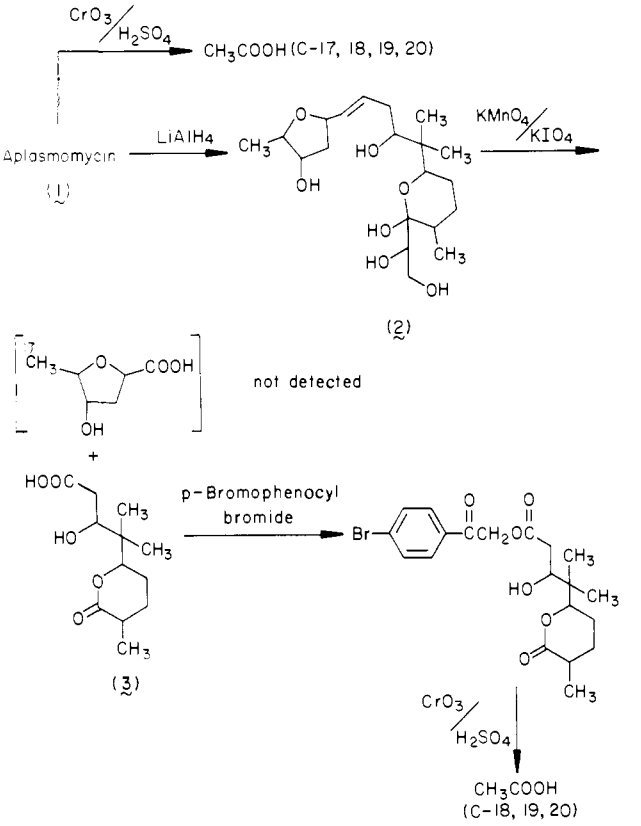
formation of the methyl group would involve the pyruvate kinase reaction which is known to proceed stereoselectively with proton addition at C-3 of phosphoenolpyruvate on the *Si* face.[22] Formation of the starter unit via methylglyoxal, on the other hand, would produce racemic methyl groups, because the methylglyoxal synthase reaction has been shown to generate a methyl group nonstereospecifically.[23] In view of the fact that **1**, in addition to C-17,17′, contains six other methyl groups, which are derived from methionine and which are also labeled by C-1 of glycerol, it was first necessary to establish with what stereochemistry the latter would be formed from a sample of chirally labeled glycerol.

To this end we fed samples of (2*S*,3*R*)-[3-$^2H_1$,^3H]serine (1.43 μCi) and (2*S*,3*S*)-[3-$^2H_1$,^3H]serine (1.09 μCi) to one culture each and isolated from each experiment about 0.5 mg of **1** with total incorporation of radioactivity of 3.9% and 3.8%, respectively. The rationale for these experiments is that the *pro-R*-hydroxymethyl group (C-1) of glycerol is converted to the hydroxymethyl group (C-3) of serine without stereochemical change and that C-3 of serine is converted to the methionine-derived methyl groups, but not to C-17,17′. Hence the chirality of the methyl groups labeled in the above experiments reflects the stereochemistry of the conversion of C-1 of glycerol into the methionine-derived methyl groups. The two samples of **1** obtained above were subjected to Kuhn–Roth oxidation,[24] and the resulting acetic acid, representing two of the three methionine-derived methyl groups in each chain, diluted with nonlabeled acetic acid from C-16/C-17, was subjected to the chirality analysis of Cornforth[25] and Arigoni[26] and their co-workers, using a procedure routinely employed in our laboratory.[27] *F* values[28] of 51.6 for the material from (3*R*)-serine and 52.7 for that from the *S* isomer indicated that the methyl groups generated are racemic. As a control and to determine the stereochemistry of the last step, the C-methylation reaction itself, we fed (*methyl-R*)- and (*methyl-S*)-[methyl-$^2H_1$,^3H]methionine (14.35 μCi, 80% ee, and 12.8 μCi, 75% ee, respectively) to five flasks each and subjected the resulting **1** samples (2.9 and 3.3 mg) to Kuhn–Roth oxidation. Chirality analysis of the acetic acid samples gave $F = 33.6$ for the sample derived from *methyl-R* methionine, indicating 57% ee *S* configuration, and $F = 65.1$ (52% e.e. R configuration) for the material from *methyl-S* methionine. Hence, the transfer of the methyl group from methionine to the polyketide chain occurs stereospecifically with inversion of configuration, and the isolation of racemic acetate is not an artefact of the analytical methodology.

It follows from the above experiments that glycerol chirally labeled at C-1 will only produce racemic methyl groups in **1** via the methionine route. Therefore, if **1** derived from such a glycerol sample is degraded by Kuhn–Roth oxidation, any enantiomeric excess in the resulting acetic acid must originate from C-17/17′. Hence, the stage was set for an experiment in which (1*R*,2*R*)-[1-$^2H_1$,^3H]glycerol (380 μCi, max 75% ee 1*R* configuration) was fed to a 5-L culture grown in a fermentor. The isolated **1**, 25 mg after purification, contained 0.88 μCi of ^3H corresponding to 0.23% incorporation. Aliquots were degraded by Kuhn–Roth oxidation, and chirality analysis of the acetate samples gave *F* values of 39.3 and 38.7 corresponding to 38% ee *S* configuration. This value represents the average of two of the three methionine-derived methyl groups plus the C-17 methyl group from each chain. From the experiments with deuteriated glycerol (Figure 1) one can estimate that C-17/17′ contributes 61% of the tritium to the acetic acid and the methionine-derived, racemic methyl groups 38%.[30]

Scheme III. Degradation of Aplasmomycin



One can therefore correct the *F* value of the acetic acid for the contribution of the racemic material, yielding a value of $F = 32$ corresponding to 61% ee *S* configuration of the methyl group of the starter unit. An attempt was made to determine this value directly. The labeled **1** from the glycerol experiment was degraded by $LiAlH_4$ reduction, followed by Lemieux–von Rudloff oxidation[31] of the resulting polyol (**2**) as shown in Scheme III. The mixture of acids obtained was derivatized with *p*-bromophenacyl bromide and the esters separated by TLC. We were unable to detect the expected fragment representing the tetrahydrofuran ring. However, fragment **3** containing only the three methionine-derived methyl groups was isolated as the bromophenacyl ester and degraded by Kuhn–Roth oxidation. The *F* values of the resulting acetic acid, 50.4 and 52.5, confirm that the chirally labeled glycerol produces methionine-derived methyl groups which are racemic. A repetition of the feeding experiment with a small sample of [1*R*,2*R*]-(1-$^2H_1$,^3H)glycerol of higher chiral purity at C-1 gave **1** which upon Kuhn–Roth oxidation produced acetic acid of $F = 35$ (52% ee *S*). Correction for the contribution of racemic methyl groups from C-18, -19, and -20 indicates that the C-17 methyl group generated in this experiment was of high chiral purity, containing 84% ee *S* configuration.

Discussion

The results reported here confirm the operation of an unusual polyketide synthesis in the formation of **1**, unusual in that it utilizes a starter unit not commonly encountered in this function and in that chain branches are introduced by C-methylation rather than by the use of homologues in place of malonyl-CoA as in normal macrolides. The stereochemistry of the C-methylation, inversion of methyl group configuration, conforms to that seen in the great majority of methyltransferase reactions studied,[27,29,32] including

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(28) The *F* value,[27,29] the percentage tritium retention in the fumarase reaction of malate generated enzymatically from chiral acetate, is a measure of the configuration and chiral purity of a chiral methyl group. Chirally pure (*R*)-acetate gives $F = 79$, pure (*S*)-acetate gives $F = 21$, and the racemate gives $F = 50$. Percent enantiomeric excess (% ee) = $\{|F - 50|/29\} \times 100$.
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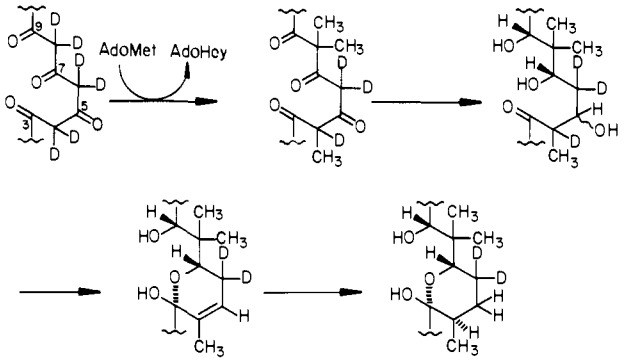
Scheme IV. Modification of the C-3 to C-9 Segment of the Polyketide Chain of **1**

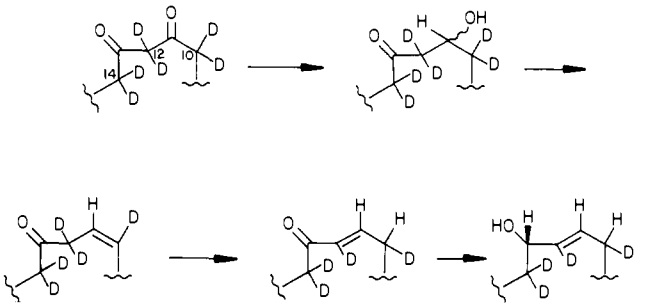


the C-methylation of indolepyruvate in the biosynthesis of indolmycin[33] and of the corrin ring system in the formation of vitamin B_{12}.[29] A C-methylation in the biosynthesis of thienamycin seems to be the only exception to this rule encountered so far.[34]

The formation of racemic methyl groups from serine chirally labeled at C-3 indicates that complete racemization must occur at this carbon atom at some stage between serine and methionine. We have studied the steric course of individual reactions in this sequence using purified enzymes. The first step, the transfer of C-3 of serine to tetrahydrofolate catalyzed by serine hydroxymethyltransferase from rat liver, proceeds with about 20% scrambling of a stereospecific tritium label at C-3 of serine between the two methylene hydrogens of 5,10-CH_2-H_4folate.[35] The last reaction, synthesis of methionine from 5-CH_3-H_4folate catalyzed by the B_{12}-dependent methionine synthase from *E. coli*, involves up to 50% racemization of the methyl group.[36] These two reactions thus account for more than half of the racemization observed, but they do not account for all of it, given that the starting serine samples were of fairly high chiral purity.[37] In unpublished work[38] we have observed that methylenetetrahydrofolate reductase from *E. coli* reduces chirally labeled 5,10-CH_2-H_4folate with formation of completely racemic methyl groups. It seems likely from the results reported here that the enzyme of *S. griseus* shows a similar lack of stereospecificity.

The data on the incorporation of methyl hydrogen and carboxyl oxygen atoms of acetate into **1** allow some suggestions as to a probable sequence of events in the modification of the original polyketide chain. Without any specific claims as to the exact order of some of the reactions, the events at the C-3 to C-9 section of the chain may be portrayed as shown in Scheme IV. Methylation, both at C-4 and C-8, at the β-diketone stage seems plausible on mechanistic grounds, and methylation at C-4 prior to removal of the C-5 oxygen via a Δ4,5-ene intermediate would account for the lack of deuterium retention at C-4. For the C-10 to C-14 segment the retention of only one atom of deuterium at C-10 implies removal of the C-11 oxygen function via a Δ10,11-ene intermediate. Since the final product contains an 11,12 double bond and C-13 initially is at the carbonyl oxidation level, a double bond shift seems a plausible possibility (Scheme V). The fact that the ^{18}O enrichment at C-1,1′ is as high as in the other labeled positions suggests that the lactone functions are formed directly

Scheme V. Modification of the C-10 to C-14 Segment of the Polyketide Chain of **1**



from a carboxyl-activated derivative released from the polyketide synthase or on the enzyme rather than from a carboxyl group obtained by hydrolysis of an activated intermediate, e.g., a thioester. In the latter case one would expect to find only half as much ^{18}O at C-1,1′ as in the other positions.

The choice of the starter unit is narrowed down by the present experiments to phosphoglyceric acid or phosphoenolpyruvate as the two most likely candidates. Most plausible alternatives are ruled out by one or several experimental observations. Serine is ruled out by the direct feeding experiment. Methylglyoxal and lactaldehyde are ruled out both by the direct feeding experiments and by the stereochemical experiment on the formation of the C-17 methyl group. The methyl group is formed stereospecifically with *S* configuration, whereas methylglyoxal synthase would have produced a racemic methyl group.[23] The stereochemical experiment also rules out pyruvate and lactate and any other metabolite formed via the pyruvate kinase reaction. The (1*R*,2*R*)-[1-$^2H_1$,^3H]glycerol fed is transformed in the cell into (*E*)-[3-$^2H_1$,^3H]phosphoenolpyruvate,[39] from which pyruvate kinase would generate a methyl group of *R* not *S* configuration.[22] Pyruvate and lactate are, in addition, also ruled out by direct feeding experiments[6] and by the experiment with [3-^{13}C]serine. The experiments with various deuteriated glycerol species define the regiochemistry of the conversion of glycerol into the starter unit. C-1, the carbon atom which is phosphorylated, gives rise to the methyl group and C-3 gives rise to C-15. The non-incorporation of deuterium from C-3 of glycerol suggests that this carbon passes through the oxidation state of a carboxyl group, as in normal polyketide biosyntheses. This rules out an unprecedented but mechanistically not unreasonable alternative, the condensation of an aldehyde as starter with malonyl-CoA to give directly the secondary alcohol function at C-15.

By process of exclusion, the available evidence thus points to phosphoglyceric acid or phosphoenolpyruvate as the most likely structures of the starter unit. Neither one has so far been encountered as a coenzyme A ester or as a polyketide chain starter unit. In view of the impermeability of cell membranes to phosphate esters a direct test of these compounds as substrates and a distinction between them will be difficult and may have to await the availability of a cell-free system. The finding that the oxygen atom of the tetrahydrofuran ring is provided by C-13 rather than by the starter unit limits the mechanistic possibilities. Using phosphoenolpyruvate as starter, one can formulate the furan ring closure as an addition–elimination sequence (Scheme VI). This process has some analogy in the 5-enolpyruvylshikimate 3-phosphate synthase reaction, and based on the recently established stereochemistry of this reaction[40-42] one can rationalize the stereochemical events leading to the formation of the chiral methyl group at C-17 as shown in the lower portion of Scheme VI. A major argument against this mechanism is the finding that the

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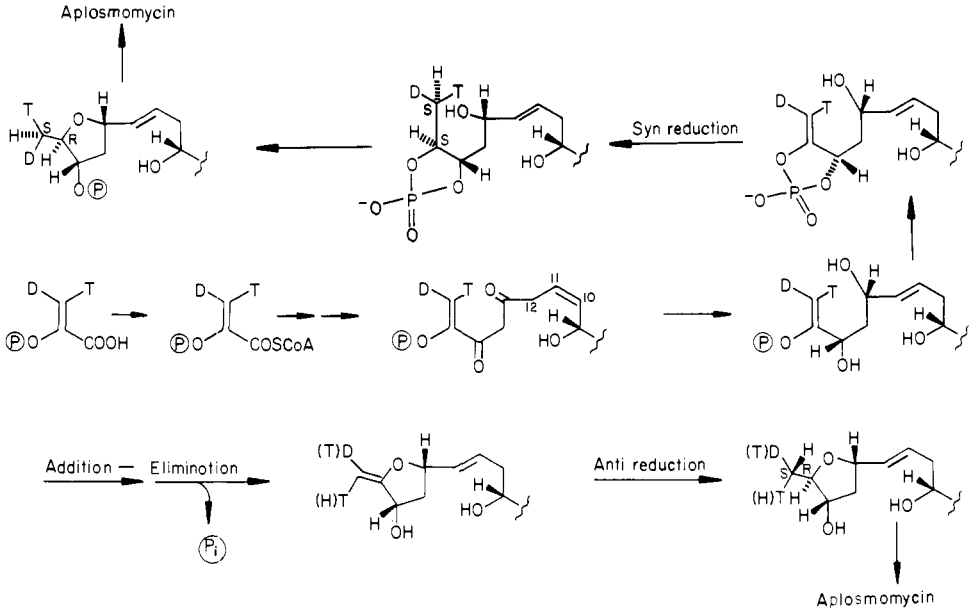
Scheme VI. Possible Mechanisms for the Initiation of the Polyketide Chain in **1** Biosynthesis by Phosphenolpyruvate



C-17,17′ methyl group is formed from (1*R*,2*R*)-[1-$^2H_1$,^{3}H]glycerol in high chiral purity (up to 84% ee). The addition–elimination process involves transient formation of a chiral methyl group from which a proton is subsequently abstracted to regenerate a methylene group.[40,41] Two methylene species will be formed, one containing tritium and deuterium (resulting from abstraction of ^{1}H), which upon reduction will give chiral methyl groups, and the other containing only tritium (resulting from abstraction of ^{2}H), which will be reduced to achiral, tritiated methyl groups (cf. ref 27). In the absence of an isotope effect in the hydrogen abstraction from the transient methyl group, the chiral purity of the C-17,17′ methyl group would be no greater than 50% ee; an isotope effect of $k_H/k_D > 5$ has to be postulated to account for the observed chiral purity of 84% ee. A second mechanism, not subject to this criticism, is shown in the top portion of Scheme VI. Here ring closure occurs by S_N2 type displacement of a phosphate, possibly a cyclic phosphate ester, from C-16 by the 13-OH group. Obviously, an analogous mechanism can be written for phosphoglycerate as the starter unit. These mechanisms are at present purely conjectural, and further experiments will be necessary to define unequivocally the nature of the starter unit and the mode of initiation of this polyketide biosynthesis.

Experimental Section

General Methods. High-field ^{1}H NMR spectra were recorded on Bruker WP 200 and WM 300 superconducting NMR spectrometers. Most ^{13}C NMR spectra were recorded on a Varian XL 200 spectrometer operating at 50.4 MHz or a Bruker WM 300 instrument operating at 75.5 MHz. ^{2}H NMR spectra were obtained on a Nicolet NC 250 NMR spectrometer operating at 38.4 MHz. Broad-band proton decoupling was employed for ^{13}C NMR and ^{2}H NMR spectra. The sample of **1** obtained from [2-^{13}C,$^2H_3$]acetate was analyzed on a modified Varian XL 100/15 spectrometer with ^{19}F lock operating at 25.16 MHz with broad-band decoupling at 100 and 15.36 MHz. Chemical shifts are given in parts per million (ppm) relative to $(CH_3)_4Si$ as internal standard or adjusted to the $(CH_3)_4Si$ scale by reference to the $CHCl_3$ resonance at 7.26 ppm or the $CDCl_3$ resonance at 76.9 ppm. Mass spectra were obtained on a Kratos MS-30 or a Finnigan 4021 mass spectrometer with either electron impact ionization at 70 eV or chemical ionization with isobutane.

Radioactive samples are counted in a Beckman LS 7000 or LS 7500 liquid scintillation counter with AQUASOL 2 (NEN) as solvent. Counting efficiencies were determined for each sample with internal standards of [^{14}C]toluene and [^{3}H]toluene.

Fermentation and Isolation of Aplasmomycin. *S. griseus* strain SS-20, a gift from Professor Y. Okami, Institute of Microbial Chemistry, Tokyo, was maintained on agar slants as described.[6] The fermentations in 500 mL shake flasks, the feeding experiments with labeled precursors, and the isolation and purification of **1** (yield 5–8 mg/L) were also carried out as previously described.[6] Larger scale production of **1** was carried out in a New Brunswick microferm fermentor with a 14-L tank. Five flasks containing 100 mL of seed culture 2 days of age were used to inoculate 10 L of fermentation medium. Air was supplied at a rate of 1.5–2 L/min and the fermentation was stirred at 300 rpm. Cultures were harvested 4 days later.

Labeled Compounds. Sodium [1-^{13}C,$^{18}O_2$]acetate (99% ^{13}C, 68% ^{18}O; 13% $^{13}C^{16}O_2$, 47% $^{13}C^{16}O^{18}O$, and 40% $^{13}C^{18}O_2$ species by NMR) was synthesized by published methods.[43–45] Sodium [2-^{13}C,$^2H_3$]acetate was purchased from Prochem Ltd., D,L-[3-^{13}C]serine from Merck Sharpe and Dohme, and [1,3-^{14}C]glycerol from Amersham-Searle. (*methyl-R*)- and (*methyl-S*)-[*methyl*-$^2H_1$,^{3}H]methionine,[33] (2*S*,3*R*)- and (2*S*,3*S*)-[3-$^2H_1$,^{3}H]serine,[37] and the (1*R*,2*R*)-[1-$^2H_1$,^{3}H]glycerol of higher chiral purity at C-1 (between 83 and 100% ee)[42] were samples available from earlier work. A quantity of (1*R*,2*R*)-[1-$^2H_1$,^{3}H]glycerol of lower chiral purity at C-1 (between 62 and 75% ee) was prepared by [^{3}H]$NaBH_4$ reduction of 2,3-isopropylidene-(*R*)-glyceraldehyde,[46] reoxidation to the aldehyde with pyridinium dichromate,[47] reduction with (−)-α-pinanyl-[$^2H_1$]-9-BBN,[48] and hydrolysis. [1,3-$^2H_4$]Glycerol and (2*R*)-[1-$^2H_2$]-glycerol were prepared by $LiAl^2H_4$ reduction of diethyl 2-acetoxymalonate and ethyl isopropylidene-(*R*)-glycerate, respectively, and hydrolysis, and [2-$^2H_1$]glycerol by NaB^2H_4 reduction of dihydroxyacetone phosphate and hydrolysis. [3-^{13}C]Methylglyoxal and D,L-[3-^{13}C]lactic aldehyde as well as the corresponding ^{14}C compounds were generated from the corresponding acetals by stirring with Dowex 50 H^+ immediately prior to each feeding experiment. 1,1-Dimethoxy[3-^{13}C]- and [3-^{14}C]propanone was prepared by condensation of methyl or butyl [2-^{13}C]- or [2-^{14}C]acetate[49] with methyl 2,2-dimethoxyacetate[50] and cleavage of the resulting β-keto ester with KOH. D,L-1,1-Diethoxy[3-^{13}C]- or [3-^{14}C]propan-2-ol was obtained by reaction of 1,1-diethoxyethanal with $^{13}CH_3MgI$ or $^{14}CH_3MgI$.[51]

Degradation of Aplasmomycin. Reduction with Lithium Aluminum Hydride. Aplasmomycin (62 mg, 0.077 mmol) and 20 mg of lithium aluminum hydride in 15 mL of anhydrous ether were stirred for 2 h at room temperature. Excess lithium aluminum hydride was destroyed by addition of cold dilute sulfuric acid to a final pH of 3.5. The product was extracted continuously with ether overnight. The ether extract was dried over anhydrous magnesium sulfate followed by purification by column chromatography (silica gel Brinkman, eluent $CHCl_3$/MeOH 9:1) to give 32 mg (53.6% yield) of a polyhydroxy compound (**2**): ^{1}H NMR (tetraacetate in $CDCl_3$, 200 MHz) δ 5.58 (1 H, m), 5.55 (1 H, m), 5.12

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(1 H, dd, J = 8.3, 2.5 Hz), 4.96 (1 H, dd, J = 9, 2.9 Hz), 4.83 (1 H, ddd, J = 6.9, 3.85, 3.5 Hz), 4.50 (1 H, dd, J = 12.3, 2.5 Hz), 4.42 (q, J = 6.8 Hz), 4.05 (1 H, dq, J = 6.4, 3.5 Hz), 3.99 (1 H, dd, J = 12.3, 8.3 Hz), 3.7 (1 H, dd, J = 12.5, 1.95 Hz), 2.99 (1 H, br s), 2.12, 2.03, 2.0, and 1.98 (4-$COCH_3$), 1.71-1.5 (4 H, m), 1.16 (3 H, d, J = 6.4 Hz), 0.94 (3 H, d, J = 5.6 Hz), 0.85 (3 H, s); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 133.6, 129.8, 98.8, 81.2, 79.4, 77.7, 77.6, 77.5, 73.6, 62.3, 40.2, 40.0, 34.3, 34.1, 27.7, 25.4, 20.9, 18.8, 16.2, 14.9.

Periodate-Permanganate Oxidation of 2. The oxidation stock solution used was freshly prepared by dissolving 448 mg of potassium periodate and 8 mg of potassium permanganate in 100 mL of distilled water with slight warming. A mixture of 30 mg of **2**, 20 mL of *tert*-butyl alcohol, and 50 mL of the oxidation stock solution was brought to pH 8.6 by addition of potassium carbonate and then stirred on a magnetic stirrer for 20 h. The mixture was acidified with 10% sulfuric acid and treated with sodium metabisulfite to convert all the periodate, iodate, and iodine into iodide. The solution was made alkaline with 5% potassium hydroxide, the butanol was distilled off, and the remaining solution was again acidified to pH 3 and continuously extracted overnight with ether. The ether extract was dried over anhydrous magnesium sulfate and then evaporated to dryness to give 16 mg of a mixture of acids. The mixture of acids was converted to the potassium salts and refluxed with 30 mg of *p*-bromophenacyl bromide and 5 mg of 18-crown-6 in 8 mL of acetonitrile for 1 h. The acetonitrile was distilled off, and the residue was purified by preparative layer chromatography (silica gel, $CHCl_3$/MeOH 95:5). Among several other degradation products, 5.7 mg of the bromophenacyl ester of the compound **3** derived from the tetrahydropyran moiety of aplasmomycin was isolated. Its structure follows from the ^1H NMR, ^{13}C NMR, and mass spectra. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.74 (2 H, d, J = 8.6 Hz), 7.61 (2 H, d, J = 8.6 Hz), 5.27 (2 H, AB q), 4.5 (1 H, dd, J = 4.4, 6.9 Hz), 3.74 (1 H, dd, J = 1.6, 4.9 Hz), 2.84 (1 H, dd, J = 4.9, 18.7 Hz), 2.7 (1 H, m), 2.57 (1 H, dd, J = 2.5, 18.7 Hz), 2.10 (1 H, m), 1.68 (2 H, m), 1.25 (3 H, d, J = 7 Hz), 1.05 (3 H, s), 0.94 (3 H, s); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 191.3, 175.6, 170.0, 133.0, 132.2, 129.9, 129.1, 81.9, 72.9, 65.6, 38.6, 36.7, 36.5, 30.0, 26.5, 21.9, 18.9, 17.2; MS (CI), $(M + H)^+$ 441.085, 443.081, calcd 441.091, 443.089.

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Registry No. 1, 61230-25-9; **2**, 109432-24-8; **3**, 109391-17-5; glycerol, 56-81-5.

Intramolecular Cyclopropanation of Enol Ethers: Synthetic Approach to Medium-Sized Carbocycles†

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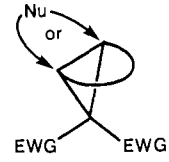
Abstract: The transannular cyclopropanation reactions of ketocarbenes, generated by $[Rh(OAc)_2]_2$ catalysis, on the 2-substituted 3,4-dihydropyran nucleus was explored. The scope and limitations of the cyclopropanation reaction were defined and where successful, and the reaction produced novel oxa-tricyclic ketones. In cases where the cyclopropanation failed, interesting and novel intramolecular C-H insertions occurred, and these could be rationalized on the basis of chain length of the diazo ketone, and substitution on the dihydropyran ring. The oxa-tricyclic ketones were subjected to acidic media and the presence of the appropriate nucleophiles and reaction conditions led to cyclopropane ring fragmentation and the formation of new carbocycles. By using this methodology, a practical approach to 6-9-membered carbocycles is described, whereby the stereochemistry of the annular substituents is controlled.

Fused and bridged polycyclic systems containing a cyclopropane ring have been shown to be useful intermediates in organic synthesis.[1] The torsional strain in the 3-membered ring imparts a high degree of reactivity, which can lead to the fragmentation of the cyclopropane. This process is hopefully a regio- and stereochemically controlled one.

One such example makes use of the homoconjugate addition of a nucleophile to a cyclopropane activated by electron-withdrawing groups (EWG). There are two important considerations associated with this approach: (a) for ring cleavage with carbon nucleophiles, the cyclopropane usually must be doubly activated by two electron-withdrawing substituents (i.e., two carbonyl functions), otherwise homoconjugate addition does not occur,[2,3] and (b) there may or may not be regiochemical discrimination in the opening of the cyclopropyl ring (Scheme I).[4]

Scheme I. Homoconjugate Addition on Activated Cyclopropanes



This paper describes the synthesis of oxa-tricyclic cyclopropyl ketones whereby both of these points are taken into consideration

† This paper is dedicated to Professor Gilbert Stork on the occasion of his 65th birthday.

‡ Merck Frosst Canada Inc.

§ Merck Sharp & Dohme Research Laboratories.

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