On the Biosynthesis of Boromycin

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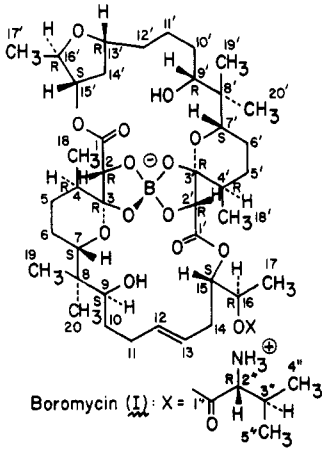
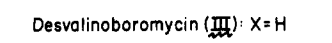
The biosynthesis of boromycin (I) in *Streptomyces sp.* was studied by feeding experiments with sodium [2-^{13}C]malonate, [*methyl*-^{13}C]methionine, and [2-^{2}H]valine followed by ^{13}C NMR and mass spectral analysis of the product. It was shown that the carbon skeleton of the macrolide portion of I is derived from 14 acetate/malonate units providing carbons 1–14 and 1′–14′, six methyl groups from methionine, giving rise to the methyl branches at carbons 4,4′ and 8,8′ of the chains, and two three-carbon starter units for the polyketide chains, which are not derived from acetate/malonate or methionine. D-Valine rather than the L isomer is the immediate precursor of the D-valyl moiety of I. The biosynthetic pathway leading to boromycin seems to parallel that for aplasmomycin.

Boromycin (I), isolated from a strain of *Streptomyces antibioticus* obtained from an African soil sample,[1] was the first natural product found to contain the trace element boron. The compound has been encountered repeatedly in antibiotic screens. For example, it was isolated independently from *Streptomyces sp.* MA 4423 at Merck Sharp and Dohme, and its use as a coccidiostat has been patented.[2] The complex structure of boromycin (I) was



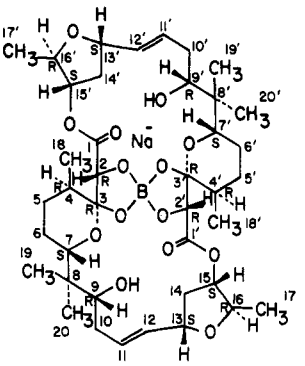
Boromycin (I): X = 1"



Desvalinoboromycin (III): X = H

elucidated by chemical degradation studies, which yielded D-valine, boric acid, and a polyhydroxy compound, and ultimately by a single-crystal, X-ray analysis of the rubidium salt of the anion obtained from boromycin by hydrolytic removal of the D-valine moiety.[3] The molecule consists of two almost identical halves which differ only in two aspects. First, a cis double bond at C-12/C-13 and an esterified hydroxy group at C-16 in one half have been modified to a tetrahydrofuran ring in the other half. Second, the configurations at C-9 and C-9′ are epimeric while all other pairs of chiral centers have the same configuration. Except for the D-valine moiety the carbon skeleton of boromycin is identical with that of aplasmomycin (II), an antibiotic recently obtained from a marine isolate of *S. griseus*.[4]

Boromycin is active against gram-positive bacteria, also certain fungi and protozoae, but is inactive against gram-negative bacteria.[3] Mode of action studies[5,6] have shown that boromycin interacts with the cytoplasmic membrane, resulting in the breakdown of the permeability barrier for potassium ions. Ability to transport ions across artificial membranes has been demonstrated for boromycin, although the specificity for potassium ion is rather low.



Aplasmomycin (II)

In this paper we report results on the biosynthesis of boromycin which show that this antibiotic is polyketide derived and that its formation probably follows the same pathway as that of aplasmomycin.

Results

The origin of the carbon skeleton of the macrocyclic dilactone ring of boromycin was studied in shake cultures of *Streptomyces sp.* MA 4423 by feeding the cultures ^{13}C-labeled precursors followed by ^{13}C NMR analysis of the product. On the basis of time course studies of boromycin formation in these cultures, the labeled precursors were added to the cultures in the production medium at about 30 h after inoculation, and incubation was continued for an additional 40–44 h. Boromycin could be isolated from the mycelium in yields of about 20 mg/L by extraction with acetone and purification by preparative layer chromatography on silica gel.

Using these conditions, the utilization of several potential precursors was evaluated in trial experiments with ^{14}C-labeled materials. The compounds fed were selected based on the structural analogy between I and II and on the established mode of biosynthesis of the latter.[7] The results, which are summarized in Table I, indicate that the

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Table I. Incorporation of Radioactively Labeled Precursors into Boromycin

precursor fed					boromycin formed				
expt	name	specif radioact, mCi/mmol	amt added, mmol/L	total radioact added, mCi/L	yield, mg/L	specif radioact of purified antibiotic, μCi/mmol	total radioact, μCi/L	incorp rate, %	specif incorp rate, %
1	[1-^{14}C]acetate	0.02	1.64	0.033	24	0.77	0.022	0.07	4
2	[2-^{14}C]propionate	0.01	2.02	0.020	22.3	0.40	0.009	0.05	3.8
3	[1-^{14}C]glycerol	0.014	1.32	0.019	20.0	0.32	0.007	0.04	2.2
4	[2-^{14}C]malonate	0.005	3.85	0.017	23.2	0.66	0.018	0.10	14.4
5	L-[$^{14}CH_3$]methionine	0.004	3.30	0.014	12.2	14.5	0.21	1.45	339
6	DL-[1-^{14}C]valine	0.01	1.98	0.010	14.8	4.95	0.68	0.068	50.8
7	D-[1-^{14}C]valine	0.014	1.30	0.015	24.3	9.8	0.27	1.83	72.9
8	L-[1-^{14}C]valine	0.013	1.36	0.018	20.0	1.7	0.039	0.22	13.1

amino acids valine and methionine are very efficiently incorporated. This suggests that the valine moiety of boromycin comes from the corresponding free amino acid and that, as in the biosynthesis of aplasmomycin, the methyl groups of methionine are utilized as building blocks. The very efficient incorporation of these two amino acids is in contrast to the rather poor utilization of acetate, propionate, or glycerol. While [1-^{14}C]acetate gave a specific incorporation of only 4% (Table I, expt 1) a somewhat better result was obtained with [2-^{14}C]malonate (Table I, expt 4). Hence, in order to probe whether the labeling pattern from these precursors and thus the mode of biosynthesis of boromycin parallels that observed for aplasmomycin, we decided to carry out feeding experiments with [*methyl*-^{13}C]methionine and [2-^{13}C]malonate.

To analyze the products from these experiments for their ^{13}C distribution, it was necessary to assign, as much as practical, the signals in the ^{13}C NMR spectrum of I to the corresponding carbon atoms. Since the objective of the study was limited to establishing whether the labeling patterns are consistent with a mode of biosynthesis of I paralleling that demonstrated for II, it was not considered essential to arrive at a completely unambiguous assignment for every signal. In particular, the very difficult distinction of corresponding carbons in the two halves of the molecule was not necessary, since the two halves, based on the aplasmomycin results, can be safely assumed to be of identical biosynthetic origin. Boromycin itself gave relatively poor ^{13}C NMR spectra, partly because of contamination with a second component which could only be removed with great losses of material.[8] However, even the spectra recorded at a field of 25.5 kG allowed an assignment of all the methyl signals, on the basis of multiplicity and chemical shift comparison with II,[9] valine,[10] and devalinoboromycin, as shown in Table II.

A better resolution of the remaining signals could be achieved by recording the spectrum of devalinoboromycin (III), obtained in good yield by refluxing I in methanol and a few drops of 20% NaOH for 1 h,[1] at a field of 90.7 kG. The proton-coupled spectrum shows 6 singlets, 13 doublets, 11 triplets, and 8 quartets (Table III). The singlets at 170.1 and 169.8 ppm, 106.0 and 105.4 ppm, 39.0 and 38.9 ppm and the doublets at 33.3 and 32.8 ppm and at 66.5 ppm were assigned to carbonyl carbons (C-1, C-1′), the quaternary carbons bearing two oxygens (C-3, C-3′), the aliphatic quaternary carbons (C-8, C-8′), the aliphatic

Table II. Tentative Assignments and ^{13}C Enrichment of the Methyl Signals in the ^{13}C NMR Spectrum of Boromycin

carbon	shift, δ	rel ^{13}C abund in I derived from [*methyl*-^{13}C]methionine[a]
19′	13.1	41.6
17	15.2	*e*
18[b]	16.5	42.0
18′[b]	16.7	43.9
4″[d]	17.8	*e*
5″[d]	18.2	*e*
17″[d]	18.9	*e*
19	19.7	43.2
20′[c]	21.4	40.2
20[c]	23.4	39.0

[a] Determined from the specific incorporation of simultaneously fed [*methyl*-^{14}C]methionine. [b-d] The signals for these carbons may be interchanged. [e] Not detectably enriched

methine carbons (C-4, C-4′), and the free hydroxy-bearing carbon C-16, respectively, on the basis of their characteristic chemical shifts and multiplicities. Of the two olefinic resonances at 133.6 and 123.6 ppm due to C-12 and C-13, the higher field one was assigned to C-13 as a consequence of the γ effect of both oxygen and carbon atoms attached to C-15. The methylene signal at highest field (20.5 ppm) was expected to belong to C-11′ because this carbon should experience considerable upfield shifts resulting from two γ-oxygen atoms attached to C-9′ and C-13′, respectively. As mentioned before, the structure of one of the two halves of devalinoboromycin containing the tetrahydrofuran ring is almost identical with each half of aplasmomycin. The spectral analysis was greatly aided by the ^{13}C resonance assignments of aplasmomycin. The signals at 12.7, (16.6, 16.3), (18.6, 18.5), (24.6, 24.9), 25.3, 27.3, (28.3, 27.8, 26.3), 30.2, (35.9, 36.7), 72.0, (85.1, 75.4), (78.2, 79.1), (76.9, 78.4, 80.0, 80.2), and 75.5 ppm could tentatively be assigned to C-19′, (C-18, C-18′), (C-17, C-17′), (C-6, C-6′), C-12′, C-10, (C-5, C-5′, C-11), C-10′, (C-14, C-14′), C-13′, (C-9, C-9′), (C-2, C-2′), (C-7, C-7′, C-15′, C-16′), and C-15, respectively, on the basis of chemical shift comparison with aplasmomycin. The quartets at 20.1, 21.0, and 23.2 ppm, by difference, must be C-19, C-20, and C-20′. Some of these assignments are not clearly distinguishable on the basis of the available data. In cases of ambiguities, the assignments listed are those compatible with the biosynthetic labeling pattern.

In the feeding experiment with ^{13}C-labeled methionine, an aqueous solution containing 500 mg of L-[*methyl*-^{13}C]methionine (90 atom % ^{13}C) and 15.5 μCi of L-[*methyl*-^{14}C]methionine (specific activity (sp ac) > 10 mCi/mmol) was added to ten 100-mL shake cultures 28

(8) The compound, which is structurally related to I, has not only been detected in our fermentations but is also present as a contaminant in every boromycin sample we obtained from other sources. It has now been purified, and its structure is being examined.

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(10) Kluender, H.; Bradley, C. H.; Sih, C. J.; Fawcett, P.; Abraham, E. P. *J. Am. Chem. Soc.* **1973**, *95*, 6149.

Table III. Tentative ^{13}C NMR Spectral Assignments[a] for Desvalinoboromycin and ^{13}C Distribution in III Biosynthesized from [2-^{13}C]Malonate

carbon	δ (ppm)	multiplicity	rel ^{13}C abund in III from [2-^{13}C]malonate
(1,1′)	(169.8, 170.1)	s	(1.1, 0.9)
(2,2′)	(79.1, 78.2)	d	(2.8, 2.8)
(3,3′)	(106.0, 105.4)	s	(1.2, 1.3)
(4,4′)	(33.3, 32.8)	d	(3.8, 3.4)
(5,5′,11)	(28.3, 27.8, 26.3)	t	(1.2, 1.2, 1.2)
(6,6′)	(24.6, 24.9)	t	(3.7, 3.8)
(7,7′,15′,16′)	(80.2, 80.0, 78.4, 76.9)	d	(1.0, 0.9, 0.9, 1.0)
(8,8′)	(39.0, 38.9)	s	(3.8, 3.8)
(9,9′)	(85.1, 75.4)	t	(1.0, 1.0)
10	27.3	t	3.0
10′	30.2	t	3.8
11′	20.5	t	0.8
12	133.6	d	3.8
12′	25.3	t	3.0
13	123.6	d	1.0
13′	72.0	d	0.9
(14,14′)	(35.9, 36.7)	t	(3.8, 3.8)
15	75.5	d	1.0
16	66.5	d	1.0
(17,17′)	(18.6, 18.5)	q	(0.8, 0.8)
(18,18′)	(16.6, 16.3)	q	(1.2, 0.8)
(19,20,20′)	(20.1, 21.0, 23.2)	q	(1.1, 1.1, 1.1)
19′	12.7	q	1.1

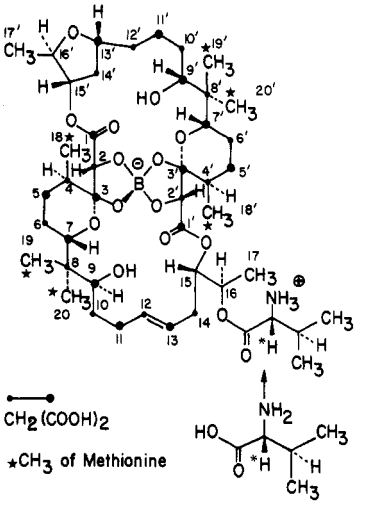
[a] Assignments within parentheses are arbitrary and may be interchanged.

h after inoculation, and the boromycin (22 mg) was isolated 44 h later. Its radioactivity (6.3×10^5 dpm total) corresponded to a specific incorporation of methionine of 243%. The ^{13}C NMR spectrum of this sample (Table II) showed that only six signals were significantly enhanced, which correspond to the methyl carbons C-18, C-18′, C-19, C-19′, C-20, and C-20′. Thus, in each of the two carbon chains of the macrolide ring of I the three branching methyl groups are derived from the methyl group of methionine, but not so the methyl groups C-17 and C-17′ at the starting point of the chains.

For the feeding experiment with malonate, we synthesized sodium [2-^{13}C]malonate from [2-^{13}C]acetic acid (90 atom % ^{13}C) without dilution with ^{12}C. An aqueous solution containing 17.2 mmol of this material (corresponding to 1.81 g of [2-^{13}C]malonic acid) and 63.6 μCi of sodium [2-^{14}C]malonate was distributed over 40 100-mL cultures 30 h after inoculation, and the boromycin (85 mg) was isolated 42 h later. The radioactivity of the product indicated a specific incorporation of 37%. For the ^{13}C NMR analysis this material was hydrolyzed to III. The well-revolved 90.5-MHz ^{13}C NMR spectrum of this sample (Table III) shows that all the even-numbered carbons from C-2 and C-2′ to C-14 and C-14′ are enriched, whereas the odd-numbered carbons and, significantly, C-16 and C-16′ show only the natural abundance ^{13}C levels. This labeling pattern agrees with that produced in aplasmomycin upon feeding with [2-^{13}C]acetate.[7]

Further studies were directed toward establishing the precursor of the D-valyl moiety in boromycin. As shown in expt 6 of Table I, DL-[1-^{14}C]valine is efficiently incorporated into I, and degradation indicated that most or all of the radioactivity resided in the D-valyl moiety. To determine whether the D or the L isomer of valine is the more immediate precursor, we compared the utilization of D- and L-[1-^{14}C]valine (Table I, expt 7 and 8). The substantially better specific incorporation of the D compared to the L form, 73% vs. 13%, suggests that the former is the more immediate precursor. However, it is still

Scheme I



possible that D-valine, although its carbon skeleton is utilized more efficiently, has to be converted to the L form before it can be used by the biosynthetic enzymes, which would then carry out another epimerization.[11] In such a process the original α-hydrogen of the amino acid would presumably be lost. We therefore prepared D- and L-[2-^2H]valine from the racemate (98 atom % ^2H) and compared their utilization in boromycin formation. Ten 100-mL cultures were used per experiment and received 133 and 123 mg, respectively, of D- or L-[1-^{14}C, 2-^2H]valine to produce 26 and 20 mg, respectively, of purified boromycin. The specific incorporations of the carbon skeleton, deduced from the radioactivity of the products, were 71% and 11%, respectively, for the D and the L isomer. Analysis of these boromycin samples by electron-impact (EI) mass spectrometry and comparison with nonlabeled material, by using the major peak at m/e 901 (boromycin sodium salt) for evaluation of the isotopic composition, indicated the presence of 42% and 0%, respectively, of singly deuterated I. This corresponds to deuterium retentions of 60% and 0% from the D and L isomers of valine; hence, D-valine is clearly the more immediate precursor of the D-valyl moiety of I. The L isomer is incorporated only via conversion to the D form with loss of the α-hydrogen.

Discussion

The results presented above establish the biosynthetic origin of boromycin as summarized in Scheme I. They indicate that the backbone of each carbon chain in the macrolide ring of I is made up from seven acetate/malonate units extending from C-1 and C-1′ as the carboxy terminus to C-14 and C-14′. Each carbon chain has undergone further modification by the introduction of three C-methyl groups derived from methionine, one at C-4 and C-4′, and two at C-8 and C-8′. This mode of biosynthesis parallels that established for aplasmomycin[7] and is in contrast to the mode of formation of the classical macrolide antibiotics, where methyl branches arise by the use of different chain extension units, e.g., propionate/methylmalonate, instead of acetate/malonate units.[13] As another

(11) In the biosynthesis of pyrrolnitrin D-tryptophan is incorporated more efficiently than the L isomer, and it alone stimulates antibiotic production. Yet D-tryptophan is incorporated with loss of and the L isomer with predominant retention of the α-hydrogen and the nitrogen of the side chain, indicating that L-tryptophan is probably the more immediate precursor.[12]

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(13) Grisebach, H. "Biosynthetic Patterns in Microorganisms and Higher Plants"; Wiley: New York, 1967.

parallel between the biosynthesis of I and that of II, the starter units, represented by C-15,C-15′, C-16,C-16′, and C-17,C-17′, are not derived from either acetate/malonate or methionine. Whether these starter units come from propionate, as one would assume a priori, or are in some way derived from glycerol, as in the biosynthesis of aplasmomycin,[7] cannot yet be decided, because both propionate and glycerol give low specific incorporations, on the same order of magnitude as acetate (Table I, expt 2 and 3). However, the close structural and biosynthetic analogy between I and II makes it a virtual certainty that the origin of the starter units in I will also turn out to be the same as that in II, i.e., from glycerol rather than from propionate. The relatively poor utilization of acetate, propionate, and glycerol in boromycin biosynthesis probably reflects a high degree of self-sufficiency of the organism in producing these compounds from the culture media ingredients rather than the distance or proximity of a precursor/product relationship. This notion is supported by the fact that malonate, although not used very efficiently either, is incorporated with a high degree of specificity. In analogy to aplasmomycin biosynthesis, we assume that propionate is only incorporated indirectly, via conversion into acetate[7] and that glycerol gives rise directly to the three carbons of the starter unit.[7]

The finding that free D-valine rather than the L isomer is the immediate precursor of the D-valyl moiety of boromycin may not seem very unusual at first glance. It is surprising, however, in view of the fact that in most secondary metabolites containing D amino acids, e.g., in various peptide antibiotics,[14] the immediate precursor is not the corresponding D but the free L amino acid; i.e., it is the L isomer which is bound by the synthetase, and epimerization at the α-carbon occurs during the process of assembly of the product.[14,15] A different mechanism is evident in boromycin biosynthesis.

Parallel and complementary results on the biosynthesis of boromycin have been obtained in the laboratory of Pape.[16]

Experimental Section

General Methods. ^{13}C NMR spectra were recorded on JEOL PFT-100 and Nicolet NTC-360 nuclear magnetic resonance spectrometers operating at 25.2 and 90.7 MHz, respectively. High-field ^1H NMR spectra were recorded at 360 MHz on a Nicolet NTC-360 instrument. Chemical shifts are given relative to Me_4Si as internal standard or adjusted to the Me_4Si scale by reference to the $CHCl_3$ resonance at δ_H 7.26 or δ_C 76.9. Mass spectra were recorded on a Varian-MAT 731 instrument.

Preparative thin layer chromatography was performed on 0.25 or 0.5 mm, 20 × 20 cm silica gel 60F-254 plates supplied by Brinkmann. The radiochemicals were used as purchased from New England Nuclear or Amersham-Searle. Radioactivity determinations were carried out by liquid scintillation counting on Beckman LS-250 and LS-7000 spectrometers using AQUASOL Universal LSC Cocktail (NEN). Counting efficiencies were determined with [^{14}C]toluene as an internal standard.

Culture Conditions. *Streptomyces sp.* strain MA 4423 was maintained on agar slants of the following medium and incubated at 28 °C until well sporulated: yeast extract, 10.0 g; glucose, 10.0 g; K, sodium phosphate buffer, 1.33 M, pH 7.0, 2.0 mL; $MgSO_4 \cdot 7H_2O$, 0.05 g; distilled H_2O, 1 L; solid agar, 20 g.

A part of the mycelial pad from a slant culture was transferred under sterile conditions to a 500-mL Bellco shake flask containing 100 mL of seed culture medium and was allowed to grow for 2–4 days on a rotary shaker at 27 °C at 300 rpm. The seed culture medium used was composed as follows: soluble starch, 10.0 g; Difco beef extract, 2.0 g; Difco yeast extract, 2.0 g; tryptose, 2.0 g; dextrose, 1.0 g; NaCl, 2.5 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCO_3$, 0.2 g; trace element solution, 10.0 mL; distilled water to 1000 mL.

The trace element solution consisted of the following: $FeSO_4 \cdot 7H_2O$, 1.0 g; $MnSO_4 \cdot 4H_2O$, 1.0 g; $CuCl_2 \cdot 2H_2O$, 25 mg; $CaCl_2$, 100 mg; H_3BO_3, 56 mg; $(NH_3)_6MoO_2 \cdot 4H_2O$, 19 mg; $ZnSO_4 \cdot 7H_2O$, 200 mg; distilled water, 1000 mL.

From this seed culture 10-mL samples were withdrawn and transferred to 500-mL Bellco shake flasks containing 100 mL of a production medium of the following composition: N-Z amine, 2.5 g; beef extract (Difco), 1.0 g; NaCl, 5.0 g; soybean meal, 10.0 g; distillers solubles, 2.0 g; corn steep liquor, 5.0 g; dextrose, 20.0 g; K_2HPO_4, 2.0 g; distilled H_2O to 1000 mL. The pH was adjusted to 7.0, followed by addition of $CaCO_3$ (10.0 g).

Isolation of Boromycin. For the isolation of boromycin, Celite (2 g per flask) was added to the cultures (pH 6.3), followed by filtration through either filter paper or three layers of cheesecloth. The mycelium was washed with water and compressed to force out most of the moisture. The mycelium was then extracted three times with 80% aqueous acetone, the extract was concentrated to a small volume, and the pH was adjusted to 5.8. This mixture was then saturated with NaCl and extracted three times with ethyl acetate; the extract was dried with sodium sulfate and evaporated to dryness in a rotary evaporator. The oily residue was dissolved in 80% aqueous methanol and defatted by extraction with 3 volumes of petroleum ether. The aqueous methanol solution was evaporated to dryness in a rotary evaporator. Boromycin was purified from the residue by preparative layer chromatography on silica gel in ethyl acetate/benzene (50:50). In the [2-^{13}C]-malonate feeding experiment the boromycin was then hydrolyzed to give devalinoboromycin as described by Hütter et al.[1]

Synthesis of [2-^{13}C]Malonic Acid. [2-^{13}C]Acetic Anhydride.[17] The reaction of dicyclohexylcarbodiimide and [2-^{13}C]acetic acid in methylene chloride yielded 90% of [2-^{13}C]acetic anhydride: bp 139 °C; ^1H NMR ($CDCl_3$) δ 2.2 (d, J = 132 Hz), 2.2 (s); CI mass spectrum, m/e (relative intensity) 103 ($M + 1^+$, 1), 104 ($M + 1^+ + 1$, 18), 105 ($M + 1^+ + 2$, 81).

[2-^{13}C]Bromoacetic Acid. This was prepared in 99% yield from [2-^{13}C]acetic acid and [2-^{13}C]acetic anhydride by bromination with bromine and red phosphorus at 120 °C: mp 50 °C; ^1H NMR ($CDCl_3$) δ 4.0 (d, J = 159 Hz), 12.1 (s); EI mass spectrum, m/e (relative intensity) 139 (M^+, 5), 141 (M^+, 5), 140 ($M^+ + 1$, 45), 142 ($M^+ + 1$, 45).

[2-^{13}C]Cyanoacetic Acid. This was prepared in 80% yield by the reaction of [2-^{13}C]bromoacetic acid with potassium cyanide: IR (Nujol) 2240, 1720 cm^{-1}; ^1H NMR (Me_2SO) δ 3.79 (d, J = 145 Hz), 3.79 (s), 11.8 (s); EI mass spectrum, m/e (relative intensity) 85 (M^+, 10.5), 86 ($M^+ + 1$, 89.5).

[2-^{13}C]Malonic Acid.[18] This was prepared by hydrolysis of [2-^{13}C]cyanoacetic acid with concentrated HCl followed by workup and recrystallization from ether (86% yield): IR (KBr) 3000, 1700 dm^{-1}; ^1H NMR (Me_2SO) δ 3.3 (d, J = 130 Hz), 3.3 (s), δ 12.0 (s); CI mass spectrum, m/e (relative intensity) 105 ($M + 1^+$, 10), 106 ($M + 1^+ + 1$, 90).

D- and L-[2-^2H]Valine. Optically pure D- and L-[2-^2H]valine were obtained by incubating DL-[2-^2H]valine with L amino acid oxidase from *Crotalus adamanteus* and D amino acid oxidase from hog kidneys, respectively, as described by Parikh et al.[19]

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Registry No. 1, 34524-20-4; 2, 61230-25-9; 3, 70658-70-7; [2-^{13}C]acetic anhydride, 77257-01-3; [2-^{13}C]bromoacetic acid, 64891-77-6; [2-^{13}C]malonic acid, 55514-11-9; D-[2-^{2}H]valine, 77257-02-4; L-[2-^{2}H]valine, 77257-03-5.

Transannular Cyclization of a *trans*-1(10)-*cis*-4-Germacradiene[1a]

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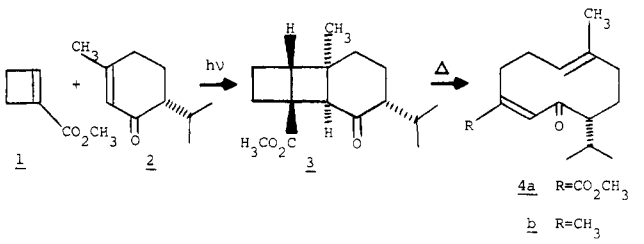
New biomimetic cyclization of the *trans*-1(10)-*cis*-4-germacradiene **4a** by using acetic acid in thiophenol afforded the cadinane **5** and the eudesmanes **6a,b** and **7**. The structure of each product was determined by spectral analysis, and the absolute configuration of **7** was determined by X-ray crystallography.

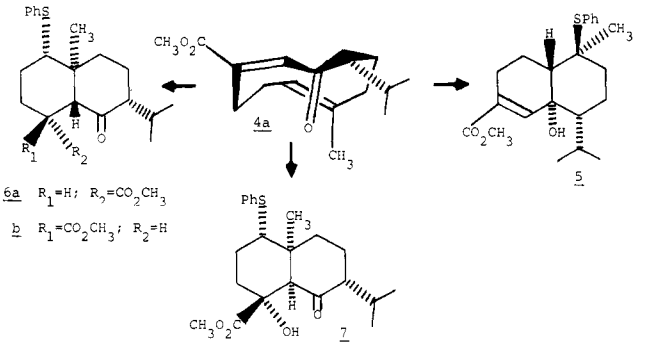
Many sesquiterpenes have been shown to be biogenetically derived from germacranes,[2] and the conversions of these 1,5-cyclodecadiene derivatives to cadinane, guaiane, and eudesmane sesquiterpenes have been the subject of several reports.[3] We felt it would be interesting to study the intramolecular cyclization reactions of the readily available synthetic *trans*-1(10)-*cis*-4-germacradiene **4a** which was synthesized in two steps from the optically active monoterpene (−)-piperitone (**2**)[1a] (Scheme I).

Treatment of **4a** with acetic acid in thiophenol gave a mixture of four isolated products which included the cadinane **5** and the three eudesmanes **6a,b** and **7** (Scheme II). The structure of the cadinane **5** was determined by the comparison of its proton and ^{13}C NMR spectra with similar synthetic and natural cadinane-type compounds (see Table I).[1] The structures of the three isomeric eudesmane products were more difficult to assign. We decided it would be useful to determine the structure of the most complex isomer **7** by X-ray crystallography. Crystal data are given in the Experimental Section. Figure 1 is a stereoscopic view of the eudesmane **7** which shows that it has a *cis*-decalone structure which contains a PhS group at C-1 and a hydroxyl group at C-4. The absolute configuration of **7** depicted in Figure 1 was determined by the anomalous scattering of the sulfur atoms and was established by refining both enantiomers. The final weighted R values were 0.0415 for the configuration shown and 0.442 for its antipode. Thus, by Hamilton's test,[4] the structure shown for **7** represents the correct absolute stereochemistry. Tables II–VI list final atomic and final anisotropic thermal parameters, bond angles, and selected torsion angles and are available as supplementary material.

Scheme I



Scheme II



The structures of **6a** and **6b** were determined by a detailed study of their proton and ^{13}C NMR spectra and by comparison to **7** and similar compounds.[3b,5]

The chemical shift of C-10 in **6a** and **6b** was considerably downfield from the chemical shift of C-10 in **7** and 10-epijunenol.[5b] This showed that the ring juncture in **6a** and **6b** was trans. A study of the molecular model of the most stable conformation of the *trans*-1(10)-*cis*-4-germacradiene **4a** and those of the products **6a** and **6b** showed that thiophenol would preferentially attack the C-1(10)-*trans*-olefin from the bottom face and would close the ring to give a *trans*-decalin and an α-axial ester at C-4, compound **6a**. The basic workup of the reaction then caused the α-axial ester at C-4 to epimerize to the thermodynamically more stable β-equatorial isomer, compound **6b**. This was confirmed by treatment of both **6a** and **6b** with

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