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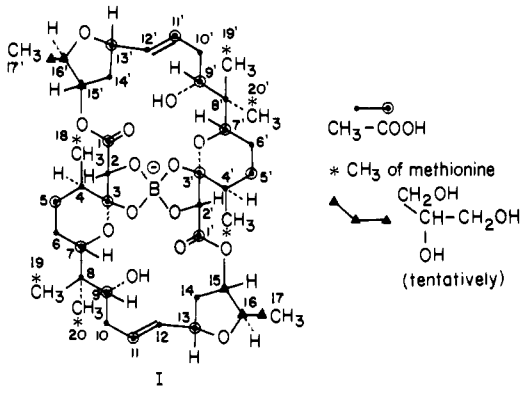
Communications to the Editor

Biosynthesis of the Boron-Containing Macrodiolide Antibiotic Aplasmomycin

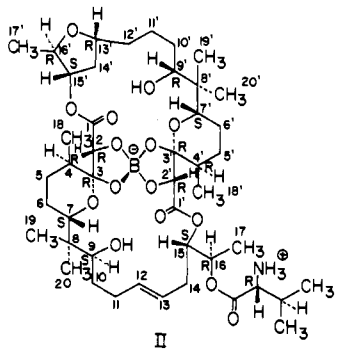
Sir:

Aplasmomycin (I) is a novel ionophoric macrodiolide antibiotic which was isolated from strain SS-20 of *Streptomyces griseus* obtained from a sample of sea mud.[1] Its structure has



I

been determined by a single-crystal X-ray analysis as a symmetric dimer built around a boron atom.[2] It is closely related to boromycin (II), the first boron-containing antibiotic found



II

in nature.[3,4] 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. In this communication, we present results on the biosynthesis of this unusual macrolide antibiotic.

Following preliminary studies with ^{14}C-labeled precursors, feeding experiments were conducted with 90% enriched sodium [1-^{13}C]-, [2-^{13}C]- and [1,2-^{13}C]acetate, and L-[*methyl*-^{13}C]methionine. The labeled precursors were added to shake cultures of *Streptomyces griseus* strain SS-20 at 48 h after innoculation, and the fermentation was continued for an additional 48 h.[5] The labeled antibiotic samples were then isolated in yields of ~10 mg/L by chloroform extraction of the broth followed by preparative TLC. The antibiotics thus obtained were analyzed by ^{13}C NMR spectroscopy.

The natural-abundance proton noise-decoupled ^{13}C NMR spectrum of aplasmomycin shows 20 signals corresponding to 40 carbon atoms of the symmetrical macrocyclic dilactone ring. Each signal represents two identical carbon atoms. An unequivocal assignment (Table I) of every signal in the spectrum was made using 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.[7]

The ^{13}C NMR spectrum of [1-^{13}C]acetate-derived aplasmomycin showed seven enhanced carbon signals representing C-1, -1′, C-3, -3′, C-5, -5′, C-7, -7′, C-9, -9′, C-11, -11′, and C-13, -13′ of the macrolide ring. Conversely, [2-^{13}C]acetate increased the intensity of the seven carbon signals corresponding to C-2, -2′, C-4, -4′, C-6, -6′, C-8, -8′, C-10, -10′, C-12, -12′, and C-14, -14′. Incorporation of 14 intact acetate units was confirmed by analysis of the antibiotic enriched by sodium [1,2-^{13}C]acetate, which showed seven pairs of doublets due to carbon–carbon coupling as characteristic satellite signals on the natural-abundance peaks. The pattern of incorporation of acetate is consistent with the polyketide pathway in the sense that the polyketide chains extend from carbon atoms 14 and 14′ through the ring system to carbon atoms 1 and 1′ in the direction of decreasing numbers of the carbon atoms with the nonacetate derived carbons 17–15 and 17′–15′ as starter units. Table I lists the relative abundance values observed in this antibiotic after feeding various precursors and the respective $^1J_{C\text{-}C}$ values found.

Three of the four methyl groups of each chain, carbons 18, 19, and 20 are derived from methionine (Table I). This is unusual since the branching methyl groups of most macrolide antibiotics, with few exceptions, e.g., the lankacidins,[8] have been demonstrated to originate from propionate units.

No significant enrichment of carbons 15, 16, and 17 was observed by any of the ^{13}C-labeled precursors employed so far. Although [2-^{14}C]- and [3-^{14}C]propionate showed good specific incorporations, 75 and 80%, respectively, into aplasmomycin, surprisingly [1-^{14}C]- and [1-^{13}C]propionate did not give any

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Table I. ^{13}C NMR Spectral Data for Aplasmomycin, Including Relative Enrichments from Labeled Precursors

carbon no.	δ_c[a]	multiplicity[b]	rel enrichment	$^1J_{C-C}$, Hz
1[c]	170.4	s	22.4[c]	64.7
2[d]	78.2	d	18.5[d]	65.2
3[c]	106.0	s	18.5[c]	47.6
4[d]	32.9	d	15.0[d]	47.6
5[c]	28.6	t	17.0[c]	31.7
6[d]	25.0	t	14.0[d]	31.7
7[c]	79.5	d	19.3[c]	39.1
8[d]	39.0	s	12.7[d]	39.1
9[c]	79.3	d	14.9[c]	39.1
10[d]	32.1	t	15.2[d]	39.1
11[c]	128.0	d	13.0[c]	72.1
12[d]	131.8	d	12.5[d]	72.0
13[c]	76.4	d	19.8[c]	34.7
14[d]	36.0	t	14.0[d]	34.7
15	80.4	d		
16	78.2	d		
17	19.4	q		
18[e]	16.5	q	56.6[e]	
19[e]	12.9	q	56.6[e]	
20[e]	21.6	q	56.6[e]	

[a] Chemical shifts are given in parts per million downfield from internal Me_4Si in $CDCl_3$. [b] Multiplicities in the off-resonance decoupled spectrum: s, singlet; d, doublet, t, triplet; q, quartet. [c] These carbon atoms were enriched by [1-^{13}C]acetate and the enrichment is relative to C-17 as 1.0. [d] These carbon atoms were enriched by [2-^{13}C]acetate and the enrichment is relative to C-17 as 1.0. [e] These carbon atoms were enriched by L-[*methyl*-^{13}C]methionine and the enrichment was estimated on the basis of the dilution of the L-[*methyl*-^{14}C]methionine fed with the ^{13}C material.

significant incorporation and enrichment. 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 propionate is not incorporated intact, but is converted, with decarboxylation, into acetate via symmetrical intermediates, i.e., succinate and the Krebs cycle. The starter unit of the polyketide, thus, does not originate from propionate.

Pyruvate, succinate, and lactate are not efficient precursors of aplasmomycin. Feeding experiments with [1,3-^{14}C]- and [2-^{14}C]glycerol gave substantial specific incorporations (18–170%). Excess cold acetate or methionine added to the same fermentation with [1,3-^{14}C]glycerol did not decrease the specific incorporation rate. Kuhn–Roth oxidation of the aplasmomycin samples derived from [1,3-^{14}C]- and [2-^{14}C]-glycerol gave sodium acetates containing 31% (of which >⅔ were located in the methyl group) and 54% of the total radioactivity, respectively. This suggests that glycerol may be specifically incorporated into the starter unit, C-1 and C-3 of glycerol probably giving rise to C-15, -15′ and C-17, -17′ of aplasmomycin, and C-2 of glycerol becoming C-16, -16′ of aplasmomycin. In view of the negative results with propionate, pyruvate, succinate, and lactate it seems possible that glycerol is incorporated into aplasmomycin via conversion to methylglyoxal as an intermediate.[10]

The biosynthetic origin of aplasmomycin can therefore be summarized as shown in I. Each half of the macrocyclic lactone ring is formed from one glycerol, seven acetate units, and three methyl groups of methionine. Further studies with [1,3-^{13}C]glycerol are in progress to determine whether the starter unit of the polyketide chain is indeed derived from glycerol.

Acknowledgments. We thank Professor Y. Okami, University of Tokyo, for kindly providing *S. griseus* SS-20 and a reference sample of aplasmomycin, Dr. K. Shimada, Hokkaido, University, for his help in procuring Kobu-cha, and Mr. J. Kozlowski of this department for recording many of the NMR spectra. This work was supported by the National Institutes of Health through Research Grant AI 11728. The services of the Purdue University Biological Magnetic Resonance Laboratory, supported by NIH Grant RR 01077 from the Division of Research Resources, are gratefully acknowledged.

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A Unique Triple Atom Bridge: X-ray Structure of the μ-Selenido-μ-diselenido-bis(tetrachloro)tungstate(V) Ion

Sir:

We believe that Y_2^{2-} units (Y = S or Se) are most easily formed by the oxidation of Y when Y bridges two metal atoms. This appears to be the case with adducts of WCl_3S. Thus the formation of such adducts by reduction of WCl_4S (which has a terminal W=S bond[1]) with excess of ligand gives an adduct in which this terminal bond remains (e.g., $WCl_3S \cdot MeSCH_2CH_2SMe$[2]). On the other hand direct reaction of WCl_3S (which contains W–S–W links) with ligands gives adducts whose infrared spectra indicate the presence of S_2^{2-} groups.[3]

As a part of our study of the chalcogenide halides WCl_3S and WCl_3Se,[4] we have examined the reactions between WCl_3Se and $(AsPh_4)Cl$ in CH_2Cl_2 solution; recrystallization of the soluble product gave brown crystals whose analysis corresponded to $(AsPh_4)_2(W_2Cl_8Se_3)$.[5]

The compound $As_2C_{48}Cl_8H_{40}Se_3W_2$ (M = 1654.34) crystallizes as brown needles in the triclinic system, space group $P\bar{1}$ with a = 12.585 (8), b = 18.151 (9), c = 14.695 (7) Å; α = 112.0 (1), β = 113.7 (1), γ = 100.3 (1)°; U = 2626.03 Å^3; D_m = 2.09 (5), D_c = 2.090 g cm^{-3}; Z = 2; μ = 86.4 cm^{-1}. The intensities of 3277 reflections (2θ < 40°) were collected manually using zirconium filtered Mo Kα radiation and the stationary crystal–stationary counter technique. Data was corrected for absorption and the 2629 reflections significantly

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