complex has been utilized in a highly efficient dihydrojasmone synthesis.[22]

(22) S. Padmanabhan and K. M. Nicholas, submitted for publication.

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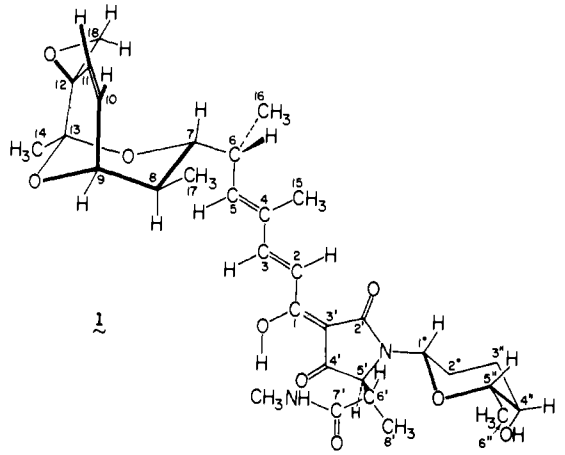
Biosynthetic Incorporation of Propionate and Methionine into Streptolydigin[1]

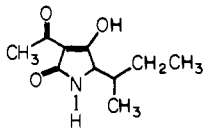
Sir:

The antibiotic streptolydigin, to which structure **1** was assigned here some years ago,[2,3] continues to be of biological interest for its potent inhibition of Gram-positive bacteria[4] and *E. coli* DNA-directed RNA polymerase[5] and its selective inhibition of terminal deoxyribonucleotidyl transferase from leukemic cells[6] and of replication of Col E1 plasmid DNA in *E. coli*.[7] Streptolydigin and the closely related tirandamycin[3,8] have also been the subjects of recent intensive studies directed toward partial[9,10] and total[11] syntheses, and closely related antibiotics continue to be discovered.[12]



1

Streptolydigin is an example of an acyltetramic acid, a class of compounds which includes the antitumor agent tenuazonic acid (**2**),[13] the toxin erythroskyrine (**3**),[14] the antitrypanosomal



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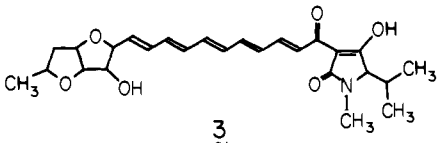
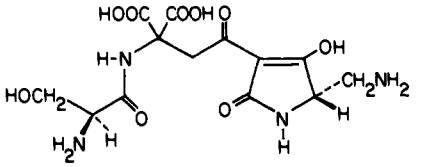


3

compound malonomicin (**4**),[15] and numerous other representatives. Biosynthesis of acyltetramic acids has been shown to involve condensation of an amino acid and a polyketide. Thus,



4

2 is derived from 1 mol of isoleucine and 2 mol of acetate,[16] **3** from valine, 1 mol of acetate, and 9 mol of malonate,[17] and **4** from 2,3-diaminopropanoic acid, acetate, a C_4 dicarboxylic acid, CO_2, and serine.[18] In principle, **1** should then be derived from β-methylaspartic acid (carbons 4′–8′), a hexose (carbons 1″–6″), and a polyketide derived *either* from acetate (carbons

Table I. Incorporation of Labeled Precursors into Streptolydigin[a]

precursor				streptolydigin			
compd	sp act., μCi/mmol	amount, μCi	total vol., mL	sp act., μCi/mmol	amount, mg	incorpn, %	% of label in streptolic acid[b]
sodium [*methyl*-^{14}C]propionate	4.8	0.16	50	0.50	3.1	1.6	100
	0.003	1.87	500	0.18	18.6	0.3	ND[c]
L-[*methyl*-^{14}C]methionine	14.9	7.08	50	11.06	1.0	0.25	2
	0.012	1.61	2000	0.20	59.0	1.25	ND
sodium [*carboxy*-^{14}C]acetate	2.2	5.84	50	0.69	1.0	0.02	ND
[*carboxy*-^{14}C]malonic acid	40	1.97	50	0.70	0.6	0.04	ND
D-[U-^{14}C]glucose	198	2.47	50	0.82	5.6	0.3	ND
DL-[1-^{14}C]glutamic acid	15.2	2.36	50	0.06	2.5	0.01	ND
DL-[4-^{14}C]aspartic acid	0.76	2.34	50	0.07	1.4	0.007	ND

precursor				streptolydigin			
compd	enrichment, % ^{13}C	amount, mg	total vol., L	enrichment, av %[d]	amount, mg	incorpn, %[e]	dilution[f]
sodium [*carboxy*-^{13}C]propionate	90	500	2.0	0.61	140	0.97	4.6
L-[*methyl*-^{13}C]methionine	60	500	2.0	0.36	164	1.56	5.2

[a] The labeled compounds were added to 50-mL aliquots of *S. lydicus* cultures in 500-mL Erlenmeyer flasks. The growth medium contained brewers yeast, 0.25%; $CaCO_3$, 0.6%; sucrose, 3%; $(NH_4)_2SO_4$, 0.2%; soybean meal, 2%; and distilled water. This was inoculated with 2.5 mL of a 72-h culture of *S. lydicus* (grown in Bacto-peptone, 0.75%; yeast extract, 0.25%; glucose, 0.5%; and distilled water) and incubated on a rotary shaker at 30 °C for 96 h. Streptolydigin was harvested by a modification of a previously reported method.[21] The culture broth was adjusted to pH 8.0 using 2 N NaOH, heated at 60 °C for 10 min and centrifuged. The supernatant was extracted using methylene chloride. The organic fraction was washed with citrate buffer and the volume reduced. Streptolydigin was precipitated by the addition of *n*-hexane, and was further purified over silica gel or using LC. [^{13}C]Streptolydigin was used directly following hexane treatment. [b] Streptolic acid was prepared from streptolydigin by periodate oxidation using the method described previously.[22] The product was purified by LC using the solvent MeOH–H_2O–AcOH (60:40:1) on a C_{18} μ-Bondapak reversed-phase column. [c] ND = not determined. [d] Calculated by summing the values in Table II and dividing by the total number of carbons to give the average ^{13}C per carbon and then subtracting natural abundance ^{13}C (taken as 1.11). [e] Calculated from ^{13}C data using enrichment values. [f] Enrichment of precursor divided by the enrichment (total) of the product.

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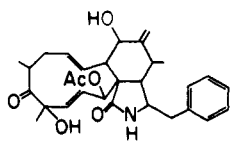
Table II. ^{13}C Abundance of Streptolydigin Carbons after Administering Labeled Precursors

streptolydigin carbon	chemical shift[a]	^{13}C abundance from individual precursors[b]	
		sodium [*carboxy*-^{13}C]-propionate	L-[*methyl*-^{13}C]-methionine
1	193.5	1.32	1.16
2	116.0	1.18	1.29
3	150.2	4.23	1.39
4	133.8	1.31	1.61
5	145.8	3.95	1.42
6	34.1	1.67	1.61
7	76.0	4.08	1.34
8	35.1	1.75	1.63
9	71.3	1.59	1.71
10	133.6	1.38	1.57
11	130.3	7.36	1.86
12	54.9	1.61	1.74
13	98.7	1.48	1.46
14	22.2	1.52	1.72
15	12.2	1.27	1.24
16	17.2	1.01	1.02
17	12.5	1.41	1.45
18	50.4	1.83	1.86
N-CH_3	26.7	1.00	5.66
2′	174.7	1.36	1.16
3′	99.6	1.07	1.08
4′	173.4	0.99	1.08
5′	62.8	1.10	1.17
6′	42.0	1.22	1.19
7′	174.8	0.81	0.85
8′	10.2	1.06	1.18
1″	78.8	0.94	0.99
2″	30.2	1.10	1.12
3″	21.3	1.17	1.16
4″	66.2	1.14	1.20
5″	76.3	0.97	0.87
6″	17.1	1.34	1.34

[a] Parts per million from Me_4Si; $CDCl_3$ solution. [b] Calculated by dividing the peak height of each signal in a particular spectrum by the average peak height (taken as 1.1) of the six deoxyhexose signals (carbons 1″, 2″, 3″, 4″, 5″, and 6″) in the same spectrum, which are assumed not to be enriched by either [*carboxy*-^{13}C]propionate or L-[*methyl*-^{13}C]methionine. For each carbon the value thus obtained from the enriched spectrum is divided by the value found for the same carbon in an unenriched spectrum.

2′, 3′; 1, 2; 9, 10; and 13, 14) and propionate (carbons 3, 4, 15; 5, 6, 16; 7, 8, 17; 11, 12, 18) *or* from acetate (carbons 2′, 3′, 1–14) and methionine (carbons 15, 16, 17, and 18). Analogy favors the latter possibility in that the modified acyltetramic acid cytochalasin D (**5**), which has a polyketide chain of the



5

same length as streptolydigin, has recently been shown to be derived from phenylalanine, 9 mol of acetate and 3 mol of methionine.[19,20] In a refutation of this analogy, we wish to report here results from a study of the biosynthesis of streptolydigin by *Streptomyces lydicus* which establish propionate rather than methionine as the source of the polyketide chain methyl groups of streptolic acid.

S. lydicus was grown on a complex medium (Table I), to which labeled potential precursors were added. Of the potential precursors added (Table I), both [*methyl*-^{14}C]propionate and [*methyl*-^{14}C]methionine were well incorporated (0.25–1.6%) into streptolydigin, while [*carboxy*-^{14}C]acetate and [*carboxy*-^{14}C]malonate were incorporated to a much lower extent (0.02–0.04%). To locate the ^{14}C, streptolydigin samples labeled by propionate and methionine were oxidized by sodium periodate to streptolic acid (C-1 through C-18),[22] which was purified by LC (Table I). [*methyl*-^{14}C]Propionate was incorporated exclusively into this part of the molecule, whereas only a very small percentage of label (2%) from methionine was found in streptolic acid (Table I). These results indicate that the branched portions of the acyl side chain of **1** are biosynthesized from propionate rather than via methionine-directed methylation of a straight chain.

To confirm this and to ascertain the position of labeling from both precursors to a more exact extent, the incorporation experiments were repeated with [*carboxy*-^{13}C]propionate and [*methyl*-$^{13}CH_3$]methionine. Examination of the ^{13}C NMR spectrum[23] of the labeled streptolydigin for enriched carbon atoms (Table II) showed that only the *N*-methyl carbon of streptolydigin is labeled by [*methyl*-^{13}C]methionine, but that [*carboxy*-^{13}C]propionate enriches C-3, C-5, C-7, and C-11 of the streptolic acid portion. These results demonstrate that four propionate residues are incorporated into the acyl side chain of streptolydigin. Acetate or malonate presumably provides carbons 13, 14; 9, 10; 1, 2; and C-2′ and C-3′ of the tetramic acid ring. Though the incorporation of [*carboxy*-^{14}C]acetate and -malonate is low, similarly relatively low incorporations have been found for other propionate-acetate polyketides.[25–28] The difference in biosynthetic pathways between streptolydigin (the product of a Streptomycete) and cytochalasin D (a fungal product) can perhaps best be interpreted in terms of Turner's observation[29] that, whereas the macrolide antibiotics (from Streptomycetes) can be derived mainly or wholly from propionate units, no such process has been observed in fungi.

The incorporations of acetate and malonate as well as the incorporations of β-methylaspartate and glucose (or another hexose) into the tetramic acid and sugar portions of streptolydigin are subjects of continuing study.

Acknowledgment. This work was supported by Public Health Service Grant AI 01278 from the National Institute of Allergy and Infectious Diseases.

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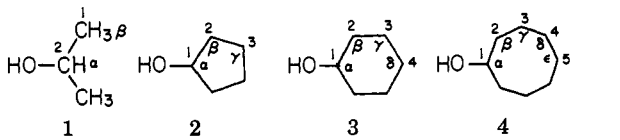
Received October 29, 1979

Additions and Corrections

Determination of the Absolute Configuration of a Secondary Hydroxy Group in a Chiral Secondary Alcohol Using Glycosidation Shifts in Carbon-13 Nuclear Magnetic Resonance Spectroscopy [*J. Am. Chem. Soc.*, **100**, 3331 (1978)]. By SHUJIRO SEO, YUTAKA TOMITA, KAZUO TORI,* and YOHKO YOSHIMURA, Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553 Japan.

On page 3333, the compound numbers and names in the structural formulas, which were not inserted in the journal, are shown below.



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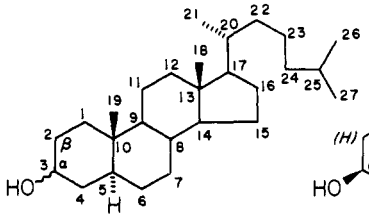
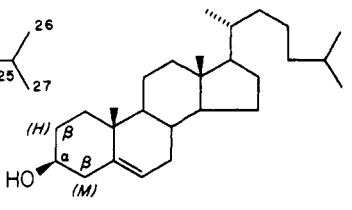
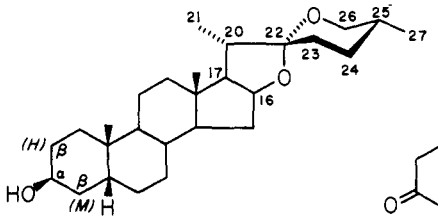


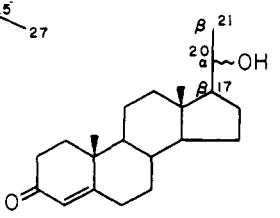
5, α-OH
6, β-OH



7, cholesterol



8, smilagenin



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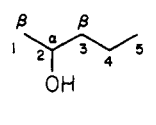
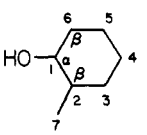
16, 20β-OH (*R*)



9, (2*R*)
10, (2*S*)



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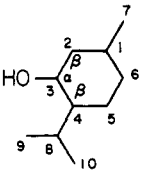
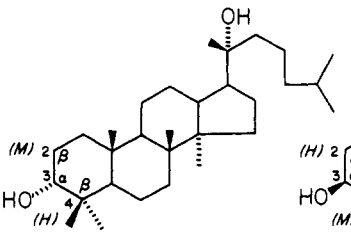
12, (1*S*)(2*S*)



13, *l*-menthol
14, *d*-menthol



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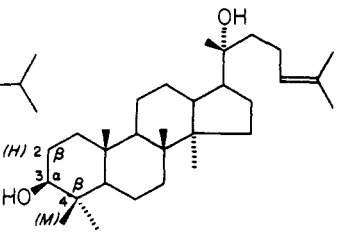

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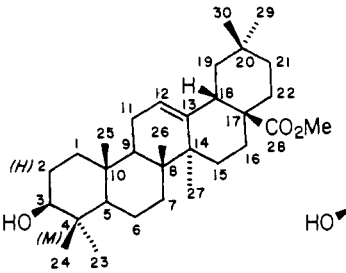
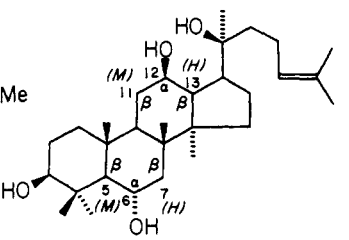


19, methyl oleanolate



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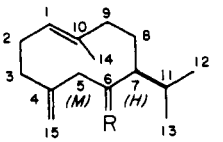
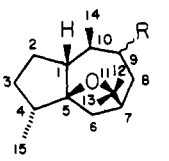
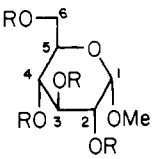


21, R = O, preisocalamendiol
22, R = 



23, R = α-OH
24, R = β-OH, liguloxidol



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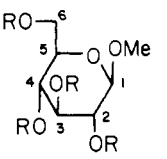
26, R = Ac



27, R = H
28, R = Ac

Calculated Infrared and Raman Spectra of the ^1A_g Ground States of Rectangular Cyclobutadiene and Tetradeuteriocyclobutadiene [*J. Am. Chem. Soc.*, **101**, 2281 (1979)]. By L. J. SCHAAD,* B. ANDES HESS, JR.,* and CARL S. EWIG,