formulation by B. Brooks and M. Karplus of Harvard University. The curve plotting software was provided to us by N. Pattabiramin at U.C.S.F. Unpublished normal modes analyses for the nucleic acids were done by D. Nguyen of U.C. Davis. A special thanks goes to Shoshana Wodak for bringing the question of protein compaction in insulin refinements to our attention and Peter Murray-Rust for carrying out the Cambridge crystal file search for phosphate geometries.

Registry No. *N*-acetyl-*N'*-methylglycinamide, 7606-79-3; *N*-acetyl-*N'*-methylalaninamide, 19701-83-8; THF, 109-99-9; MEE, 540-67-0; dimethyl phosphate, 813-78-5; diethyl phosphate, 598-02-7; deoxyadenosine, 958-09-8; adenosine, 58-61-7; 9-methylguanine, 5502-78-3; 1-methylcytosine, 1122-47-0; 9-methyladenine, 700-00-5; 1-methylthymine, 4160-72-9; 1,3-dimethyluracil dimer, 40037-86-3; poly[d(G-C)], 36786-90-0; poly d(G)·poly d(c), 25512-84-9; poly[d(A-T)], 26966-61-0; poly d(A)·poly d(T), 24939-09-1; poly[d(T-G)]·poly[d(C-A)], 27732-52-1; poly[d(T-C)]·poly[d(G-A)], 29627-66-5; poly[d(A-T-C)]·poly[d-(G-A-T)], 24939-08-0; poly[d(T-T-G)]·poly[d(C-A-A)], 27902-32-5; poly[d(T-A-C)]·poly[d(G-T-A)], 57473-66-2; poly[d(T-T-C)]·poly[d(G-A-A)], 27861-08-1; glycylglycine, 556-50-3; alanylalanine, 1948-31-8; benzene, 71-43-2; *N*-methylacetamide, 79-16-3; methanol, 67-56-1; methanethiol, 74-93-1; dimethyl sulfide, 75-18-3; dimethyl disulfide, 624-92-0; insulin, 9004-10-8; Gly, 56-40-6; Thr, 72-19-5; Ile, 73-32-5; Ser, 56-45-1; Leu, 61-90-5; Val, 72-18-4; Asn, 70-47-3; Arg, 74-79-3; Gln, 56-85-9; Ala, 56-41-7; Phe, 63-91-2; Tyr, 60-18-4; Cys, 56-89-3; Met, 63-68-3; Hie, 71-00-1; Hip, 70805-60-6; Gln, 56-86-0; Trp, 73-22-3; Asp, 56-84-8; Lys, 56-87-1; Pro, 147-85-3; adenine, 73-24-5; guanine, 73-40-5; thymine, 65-71-4; cytosine, 71-30-7; uracil, 66-22-8.

Communications to the Editor

Biosynthetic Origin of the Carbon Skeleton and Oxygen Atoms of Nargenicin A_1

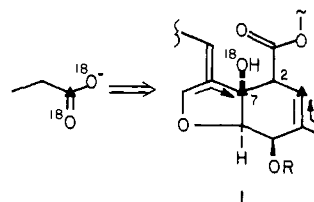
David E. Cane*[1] and Chi-Ching Yang

Department of Chemistry, Brown University Providence, Rhode Island 02912

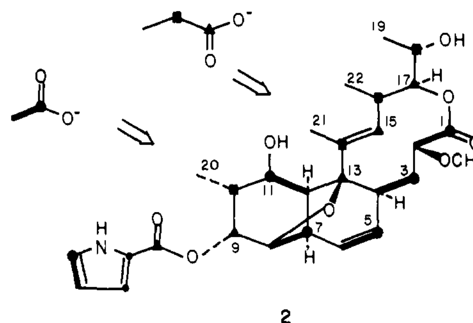
Received September 21, 1983

It is well accepted that in the biosynthesis of aromatic polyketides the fundamental chain-building and ring-forming reactions take place by similar carbonyl condensation mechanisms.[2] An analogous, albeit far more complex, sequence of reactions is also believed to be responsible for the formation of reduced linear polyketides typified by the macrolide[3] and polyether[4] antibiotics. Thus recent studies of erythromycin,[5] monensin,[6] and lasalocid[7] biosynthesis have supported the notion that these polyoxygenated, branched-chain fatty acids are assembled by a sequence of condensation, reduction, dehydration, and reduction reactions closely related to those leading to classical saturated fatty acids. The biosynthesis of reduced, carbocyclic polyketides, on the other hand, is far less well understood. Although the parent polyketide chains, in all cases examined to date, have been shown to be derived largely from the common precursors acetate and propionate, little evidence is available to allow a distinction among plausible carbonyl condensation, electrophilic polyolefin cyclization, and Diels-Alder sequences that might account for the formation of the characteristic carbocyclic ring systems.[8] Recently, as part of a study

Scheme I



Scheme II



of avermectin (**1**) biosynthesis, we presented evidence suggesting that the C-2,7 bond of the constituent cyclohexene ring is probably generated by condensation of a C-7 carbonyl group with a carbonyl-stabilized anion.[9] Specifically, the observed derivation of the C-7 hydroxyl group from the carboxylate oxygens of the propionate precursor ruled out the alternative cyclization of a polyolefinic intermediate (Scheme I). We have now extended our studies to an examination of the biosynthesis of the saturated cyclic polyketide nargenicin A_1 (**2**) an antibiotic active against *Staphylococcus aureus* and containing a novel octalin ring system.[10,11]

(1) National Institutes of Health Research Career Development Award, 1978-1983.

(2) Weiss, U.; Edward, J. M. "The Biosynthesis of Aromatic Compounds"; Wiley: New York, 1980; pp 326-428. Herbert, R. B. "The Biosynthesis of Secondary Metabolites"; Chapman and Hall: London, 1981; pp 28-49.

(3) Corcoran, J. W. In "Antibiotics IV. Biosynthesis"; Corcoran, J. W., Ed.; Springer-Verlag: New York, 1981; pp 132-174. Omura, S.; Nakagawa, A. *Ibid.* pp 175-192.

(4) Westley, J. W., ref 3, pp 41-73. Liu, C.-M. In "Polyether Antibiotics. Naturally Occurring Acid Ionophores"; Westley, J. W., Ed.; Marcel Dekker: New York, 1982; Vol 1, pp 43-102.

(5) Cane, D. E.; Hasler, H.; Liang, T.-C *J. Am. Chem. Soc.* **1981**, *103*, 5960. Cane, D. E.; Hasler, H.; Taylor, P. B.; Liang, T.-C. *Tetrahedron* **1983**, *39*, 3449.

(6) Cane, D. E.; Liang, T.-C; Hasler, H. *J. Am. Chem. Soc.* **1981**, *103*, 5962; **1982**, *104*, 7274. Ajaz, A. A.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1983**, 645.

(7) Hutchinson, C. R.; Sherman, M. M.; Vederas, J. C.; Nakashima, T. T. *J. Am. Chem. Soc.* **1981**, *103*, 5953. Hutchinson, C. R.; Sherman, M. M.; McInnes, A. G.; Walter, J. A.; Vederas, J. C. *Ibid.* **1981**, *103*, 5956.

(8) Besides the bicyclic octalin metabolites discussed in this report, members off the class of reduced carbocyclic polyketides include, inter alia, monocyclic cyclopentanes (e.g., brefeldin A: Haerri, E.; Loeffler, W.; Sigg, H. P.; Staehlin, H.; Tamm, Ch. *Helv. Chim. Acta* **1963**, *46*, 1235. Sigg, H. P. *Ibid.* **1964**, *47*, 1401), monocyclic cyclohexanes (palitantin: Bowden, K.; Lythgoe, B.; Marsden, D. J. S. *J. Chem. Soc.* **1959**, 1662), bicyclic hydrindanes (antibiotic X-14547A: Westley, J. W.; Evans, R. H.; Liu, C.-M; Hermann, T.; Blount, J. F. *J. Am. Chem. Soc.* **1978**, *100*, 6784), and *as*-hydrinacenes (ikarugamycin: Ito, S.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1813.)

(9) Cane, D. E.; Liang, T.-C.; Kaplan, L.; Nallin, M. K.; Schulman, M. D.; Hensens, O. D.; Douglas, A. W.; Albers-Schoenberg, G. *J. Am. Chem. Soc.* **1983**, *105*, 4110.

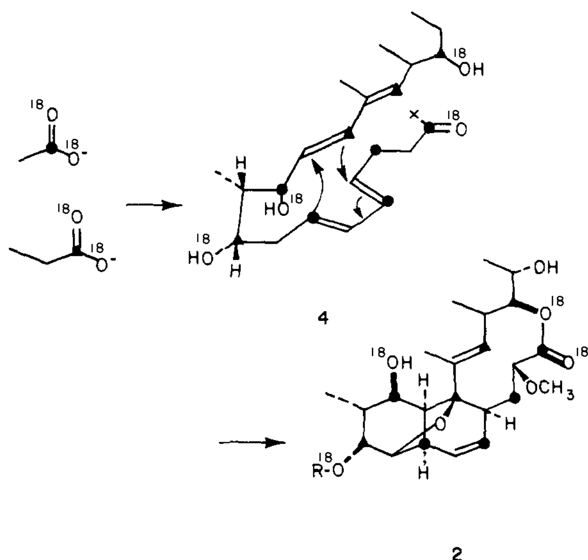
0002-7863/84/1506-0784$01.50/0 © 1984 American Chemical Society

Table I. ^{13}C NMR Spectrum of Nargenicin A_1 and Incorporation of ^{13}C-Labeled Precursors

chemical shift, δ (m)[a]	C	precursors[b]: [1-^{13}C]-acetate[c]	[2-^{13}C]-acetate[c]	[1,2-$^{13}C_2$]-acetate[c] *J*, Hz	[1-^{13}C]-propionate[d]	[2-^{13}C]-propionate[d]
172.6 (s)	1	*		61.7		
83.4 (d)	2		*	61.7		
34.4 (t)	3	*		38.0		
43.1 (d)	4		*	38.0		
132.9 (d)	5	*		67.6		
127.5 (d)	6		*	67.6		
39.1 (d)	7	*		31.5		
81.3 (d)	8		*	31.5		
73.7 (d)	9				*	
35.1 (d)	10					*
76.2 (d)	11	*		37.0		
49.1 (d)	12		*	37.0		
89.1 (s)	13				*	
134.3 (s)	14					*
131.5 (d)	15				*	
32.8 (d)	16					*
78.9 (d)	17				*	
66.6 (d)	18					*
20.7 (q)	19					
13.0 (q)	20					
17.0 (q)	21					
15.6 (q)	22					
57.6 (q)	23					
160.4 (s)	1'				*	
122.4 (s)	2'					
115.2 (d)	3'					
110.4 (d)	4'		*	63.4		
123.4 (d)	5'	*		63.4		

[a] $CDCl_3$, 62.9 MHz; spectral width 19 231 Hz. 32K data points; quadrature detection; 30° pulse; repetition rate 1.1 s. [b] Sites of enrichment indicated by * or J_{CC} coupling constants. [c] Average ^{13}C enrichment, nargenicin nucleus, 5–7%; pyrrolecarboxylate, 5–6%. [d] Average ^{13}C enrichment, nargenicin nucleus, 7–10%; pyrrolecarboxylate, 2%.

Scheme III



The basic building blocks for nargenicin were established by a series of straightforward incorporation experiments employing [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetate and [1-^{13}C]- and [2-^{13}C]propionate. These precursors were administered to actively fermenting cultures of *Nocardia argentinensis* Huang, ATCC 31306, and the resulting labeled samples of nargenicin were analyzed by 62.9-MHz ^{13}C NMR. The results, which are fully in accord with the expected derivation of nargenicin from five acetates and four propionates, are summarized in Table I and in Scheme II.[12,13] An interesting sidelight was the observed labeling of the pyrrole carboxylate moiety by acetate and propionate with a pattern consistent with conversion of propionate to succinate and thence to dehydroproline via α-ketoglutarate.[14,15]

(10) Celmer, W. D.; Chmurny, G. N.; Moppett, C. E.; Ware, R. S.; Watts, P. C.; Whipple, E. B. *J. Am. Chem. Soc.* **1980**, *102*, 4203. Celmer, W. D.; Cullen, W. P.; Moppett, C. E.; Jefferson, M. T.; Huang, L. H.; Shibakawa, R.; Tone, J. U.S. Patent 4 148 883, 1979.

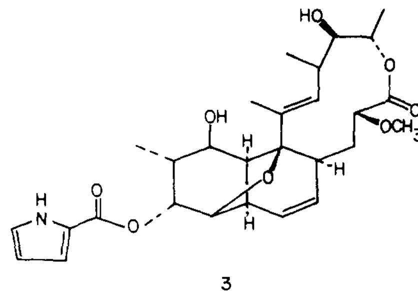
(11) Nargenicin is closely related to nodusmicin, a metabolite of *Saccharopolyspora hirsuta*, which differs from nargenicin only by the presence of an unesterified hydroxyl group at C-9 in place of the pyrrolecarboxylate ester: Whaley, H. A.; Chidester, C. G.; Mizsak, S. A.; Wnuk, R. J. *Tetrahedron Lett.* **1980**, *21*, 3659. Among other minor derivatives of nargenicin that have been reported is 18-deoxynargenicin A_1: Whaley, H. A.; Coats, J. H. *Abstr. Interscience Conf. Antimicrob. Agents Chemother.* **1981**, *No. 187.*

(12) The ^{13}C NMR spectrum of nargenicin A_1 has previously been assigned.[10] On the basis of a study of the spectra of nargenicin 9,18-diacetate and the corresponding 9- and 18-monoacetate esters, we have concluded that the earlier assignments of C-7 and C-12 should be reversed. These revisions are borne out by the labeling results reported herein.

(13) Some unexceptional, indirect labeling of propionate-derived carbon atoms by acetate was also observed.

(14) Zmijewski has reported that the acylpyrrole moiety of calcimycin is derived from proline: Zmijewski, M. J. *J. Antibiot.* **1980**, *33*, 447. Zmijewski, M. J.; Wong, R.; Paschal, J. W.; Dorman, D. E. *Tetrahedron* **1983**, *39*, 1255. See also: David, L.; Emadzadeh, S. *J. Antibiot.* **1982**, *35*, 1616.

(15) In all of these feeding experiments, we also isolated small quantities of a nargenicin cometabolite which was unambiguously assigned structure **3**



on the basis of high field ^{1}H and ^{13}C NMR analysis as well as high-resolution mass spectrometry (*m/e* 515.2538; calcd for $C_{28}H_{37}NO_8$: 515.2519). The same ring-expanded lactone, which we have named isonargenicin, has previously been obtained by Celmer et al.[10] upon treatment of **2** with base. Whether **3** is a true natural product or simply a rearrangement product of **2** has yet to be established. Nonetheless, samples of **3** isolated from each ^{13}C-incorporation experiment displayed labeling patterns identical with those observed for the nargenicin. These results are summarized in Table II.

Table II. ^{13}C NMR Spectrum of Isonargenicin (3) and Incorporation of ^{13}C-Labeled Precursors

		precursors[b]				
chemical shift, δ (m)[a]	C	[1-^{13}C]-acetate[c]	[2-^{13}C]-acetate[c]	[1,2-$^{13}C_2$]-acetate[c] J, Hz	[1-^{13}C]-propionate[d]	[2-^{13}C]-propionate[d]
171.4 (s)	1	*		61.6		
83.4 (d)	2		*	61.6		
33.0 (t)	3	*		37.6		
41.9 (d)	4		*	37.6		
132.6 (d)	5	*		67.9		
127.4 (d)	6		*	67.9		
38.8 (d)	7	*		31.6		
81.5 (d)	8		*	31.6		
73.8 (d)	9				*	
35.0 (d)	10					*
76.1 (d)	11	*		37.1		
49.5 (d)	12		*	37.1		
88.8 (s)	13				*	
131.0 (s)	14					*
131.6 (d)	15				*	
39.6 (d)	16					*
76.4 (d)	17				*	
74.3 (d)	18					*
18.5 (q)[e]	19					
12.7 (q)	20					
16.8 (q)	21					
18.4 (q)[e]	22					
57.3 (q)	23					
160.4 (s)	1'				*	
122.4 (s)	2'					
115.2 (d)	3'					
110.4 (d)	4'		*	63.5		
123.3 (d)	5'	*		63.5		

[a] $CDCl_3$, 62.9 MHz; spectral width 19231 Hz. 32K data points; quadrature detection; 30° pulse; repetition rate 1.1 s. [b] Sites of enrichment indicated by * or J_{CC} coupling constants. [c] Average ^{13}C enrichment, nargenicin nucleus, 5–7%; pyrrolecarboxylate, 5–6%. [d] Average ^{13}C enrichment, nargenicin nucleus, 7–10%; pyrrolecarboxylate, 2%. [e] These assignments may be interchanged.

Table III. Incorporation of [1-^{13}C,1-$^{18}O_2$]Acetate and [1-^{13}C,1-$^{18}O_2$]Propionate into Nargenicin A_1

precursor							
[1-^{13}C,1-$^{18}O_2$]acetate				[1-^{13}C,1-$^{18}O_2$]propionate			
C	^{13}C shift, ppm[a]	Δδ[c]	^{18}O/^{16}O[d]	C	^{13}C shift, ppm[b]	Δδ[c]	^{18}O/^{16}O[d]
1	172.62	0.04	75:25	9	73.88	0.03	45:55
3	34.39			13	89.21	0.00	0:100
5	132.93			15	131.59		
7	39.13			17	79.07	0.03	65:35
11	76.31	0.02	75:25				
17[d]	78.93	0.03	15:85				

[a] Bruker WM-250, 62.9 MHz; spectral width 12 195 Hz; 128K points; quadrature detection; 30° pulse; repetition rate 1.34 s; resolution enhancement by Lorentz-Gauss multiplication of FID prior to Fourier transformation; −0.8-Hz line broadening, 0.4 Gaussian multiplier; 0.003 ppm/data point. [b] Acquisition parameters identical with acetate-derived sample; −1.2-Hz line broadening, 0.4 Gaussian multiplier; 0.003 ppm/data point. [c] $^{13}C^{18}O$ isotope shift; ±0.003 ppm. [d] ±5; uncorrected for contribution of natural abundance ^{13}C to $^{13}C^{16}O$ peak. [e] Indirect labeling via conversion to propionate.

Having confirmed the identity of the primary precursors of nargenicin and having established suitable experimental conditions for efficient incorporation of ^{13}C-labeled substrates, we turned our attention to the determination of the biosynthetic origin of the oxygen atoms of nargenicin. We therefore carried out incorporations of [1-$^{18}O_2$,1-^{13}C]acetate[16] and [1-$^{18}O_2$,1-^{13}C]-propionate[17] and analyzed the resulting nargenicin in each case by ^{13}C NMR (Table III). On the basis of the observation of characteristic ^{13}C–^{18}O isotope shifts[5–7,9,18] in the spectra of the derived nargenicin samples, the oxygen atoms at C-1 and C-11 of **2** were shown to originate from the carboxylate oxygens of the acetate precursor, while the C-9 and C-17 oxygen atoms were found to be derived from propionate. The remaining nargenicin oxygen atoms, at C-2, C-8,13, and C-18 are presumably derived from molecular oxygen, a question that is currently under investigation.

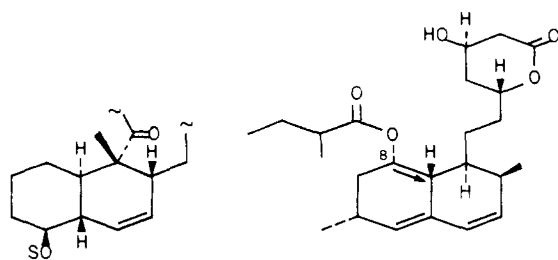
The established derivation of the ether and carbonyl oxygen atoms of the lactone from the distinct propionate and acetate subunits is in keeping with earlier observations of macrolide biosynthesis.[5,9,19] The fact that neither the C-9 nor the C-11 oxygens of **2** are derived from molecular oxygen rules out various hypothetical ring-forming mechanisms based on epoxy olefin and epoxy alcohol cyclizations. On the other hand the absence of propionate-derived oxygen at C-13 strongly suggests that the corresponding C-4,13 bond has not been formed by an aldol-type condensation analogous to that thought to be responsible for cyclohexene ring formation in the biosynthesis of avermectin. An attractive alternative, which accounts for the observed distribution of isotopic labels and which is consistent with the stereochemistry and functional group distribution in nargenicin, is an intramolecular Diels–Alder reaction between an E,E 4,6-diene and an E dienophile (**4**) (Scheme III). The dienophile might be activated by conjugation with the adjacent C-14 double bond or with a

(16) 73.4% $^{18}O_2{}^{13}$C, 14.6% $^{18}O^{13}$C, 0.8% $^{16}O^{13}$C. Prepared as previously described.[4,5]

(17) 54.9% $^{18}O_2{}^{13}$C, 32.2% $^{18}O^{13}$C, 3.6% $^{16}O^{13}$C. Prepared as previously described.[4,5]

(18) Vederas, J. C. *J. Am. Chem. Soc.* **1980**, *102*, 374. Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.*, **1980**, *101*, 252; *Ibid.* **1980**, *102*, 4609, 6699.

carbonyl function at C-9. It is interesting to note that closely related mechanisms can account for the formation of the octalin ring systems in chlorothricin (**5**),[20] kijanimicin,[21] and tetrocarcin,[22]



5 6

all of which exhibit the identical cis relationship between the H-4 and H-7 hydrogen atoms (nargenicin numbering) and retain a carbonyl group adjacent to the presumptive dienophile.[23]

Fermentation of *Nocardia argentinensis* and Incorporation of Labeled Precursors.[10] An inoculum of *Nocardia argentinensis* Huang, ATCC 31306, was prepared by transfer of cells from a slant culture to a 500-mL Delong flask containing 70 mL of vegetative medium consisting of 10.0 g of glucose, 20.0 g of starch, 5.0 g of yeast extract, 5.0 g of enzymatic digest of casein, 0.5 g of dipotassium hydrogen phosphate, 5.0 g of meat meal, 0.002 g of $CoCl_2$, and 4.0 g of $CaCO_3$ per L of distilled water, final pH 7.1-7.2. The vegetative culture was incubated at 250 rpm and 28 °C for 4 days before being used to inoculate (3% v/v) a series of 500-mL Delong flask each containing 70 mL of a fermentation medium composed of 1.0 g of glucose, 2.5 g of enzymatic digest of casein, 5.0 g of soluble starch, 5.0 mL of corn steep liquor, 3.0 g of $CaCO_3$, and 0.002 g of $CoCl_2$ per L of distilled water, final pH 6.9-7.0. The cultures were incubated at 30 °C and 250 rpm for 4 days before extraction and isolation of antibiotic.

For feeding experiments, labeled precursors were dissolved in distilled water and added in three portions (40%, 30%, and 30%) through a disposable sterile filtration unit to each fermentation flask after 24, 48, and 72 h, respectively, during the normal fermentation period. The total dose of sodium acetate and propionate was 3.0 g/L in each case. The 90% ^{13}C-enriched acetate and propionate precursors were diluted prior to feeding with two and five parts, respectively, of unlabeled precursor, in order to avoid excess intramolecular multiple labeling in the isolated metabolites.

After 4 days, the mycelia and fermentation broth were separated by centrifugation for 15 min at 12000*g* and each was extracted with several portions of chloroform. Concentration of the combined organic extracts gave an orange residue, which was washed and filtered through a Celite pad with 300-500 mL of ethyl acetate. After evaporation of the ethyl acetate, the residue was subjected to purification by preparative TLC (ethyl acetate-cyclohexane, 1:1; nargenicin, R_f 0.27; isonargenicin, R_f 0.36). The recovered nargenicin was further purified by TLC (ethyl ether-benzene, 1:1). Typical yields were 20-30 mg/L of nargenicin and 5-7 mg/L of isonargenicin.

Acknowledgment. This work was supported by a grant from the NIH, GM 22172. The strain of *N. argentinensis* as well as an authentic sample of nargenicin A_1 were kindly provided by Dr. Walter D. Celmer and Paul Watts of Ch. Pfizer, Inc., who also furnished helpful information on fermentation and isolation conditions. The Bruker WM 250 NMR used in this work was purchased with funds provided by the NSF and the Montedison Group of Milan.

Registry No. 2, 70695-02-2; **3**, 74666-93-6; acetic acid, 64-19-7; propionic acid, 79-09-4; carbon, 7440-44-0; oxygen, 7782-44-7.

(19) Mabuni, C. T.; Garlaschelli, L.; Ellison, R. A.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1979**, *101*, 707.

(20) Structure: Muntwyler, R.; Keller-Schierlein, W. *Helv. Chim. Acta* **1972**, *55*, 2071. Biosynthesis: Holzbach, R.; Pape, H.; Hook, D.; Kreutzer, E. F.; Chang, C.-J.; Floss, H. G. *Biochemistry* **1978**, *17*, 556. Floss, H. G.; Chang, C.-J.; Mascaretti, O.; Shimada, K. *Planta Med.* **1978**, *34*, 345. Mascaretti, O.; Chang, C.-J.; Floss, H. G. *J. Nat. Prod.* **1979**, *42*, 455.

(21) Structure: Mallams, A. K.; Puar, M. S.; Rossman, R. R. *J. Am. Chem. Soc.* **1981**, *103*, 3940. Mallams, A. K.; Puar, M. S.; Rossman, R. R.; McPhail, A. T.; Macfarlane, R. D.; Stephens, R. L. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1497.

(22) Structure: Tomita, F.; Tamaoki, T.; Shirahata, K.; Kasai, M.; Morimoto, M.; Ohkubo, S.; Mineura, K.; Ishii, S. *J. Antibiot.* **1980**, *33*, 668. Hirayama, N.; Kasai, M.; Shirahata, K.; Ohashi, Y.; Sasada, Y. *Tetrahedron Lett.* **1980**, *21*, 2559. Biosynthesis: Tamaoki, T.; Tomita, F. *Ibid.* **1983**, *36*, 595.

(23) The fungal metabolite mevinolin (**6**), whose biosynthesis has recently been studied by Vederas (Chan, J. K.; Moore, R. N.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 3334), presents a more subtle mechanistic problem. The presence of an additional conjugated double bond within the hexalin ring system masks the original stereochemistry at the bridgehead, while the fact that the carbinyl hydrogen atom at C-8 (corresponds to C-11 of nargenicin) is derived from an acetate methyl hydrogen rules out the intermediacy of a carbonyl function at this site.

Biosynthesis of Nargenicin and Nodusmicin

William C. Snyder and Kenneth L. Rinehart, Jr.*

Roger Adams Laboratory, University of Illinois
Urbana, Illinois 61801

Received September 21, 1983

The antibiotics nodusmicin (**1**) and nargenicin (**2**) represent a novel group of macrolide antibiotics recently isolated from fermentations of *Saccharopolyspora hirsuta* strain 367 (NRRL 12045)[1] and *Nocardia argentinensis* Huang (ATCC 31306),[2] respectively. Related compounds belonging to this reduced polyketide class have since been reported, along with additional strains producing the antibiotics.[3,4] Significant activity against anaerobes and resistant strains of *Staphylococci*, coupled with low toxicity and substantial oral activity, has led to an extensive analogue program at major pharmaceutical laboratories.[5-7]

The biosynthetic origin of the family is of particular interest due to the antibiotics' octahydronaphthalene ring system, as well as to their commercial potential. In principle, the macrolide ring could be derived from the typical *Actinomycete* acetate-propionate pathway,[8] although the acetate-methionine pathway of fungi[9] is also plausible. The oxygens at C-11, C-13, and C-17 are appropriately placed for either pattern. The C-2 oxygen could indicate a glycolate origin for the C-1,C-2 unit as in geldanamycin[10] and leucomycin (via glycerol),[11] or it could be introduced at an acetate-derived carbon. The pyrrole carbonyl unit is presumably derived from glutamate via proline.[12]

In the present work, fermentation studies designed to optimize production of nargenicin from *N. argentinensis* led to the detection

(1) Whaley, H. A.; Chidester, C. G.; Mizsak, S. A.; Wnuk, R. J. *Tetrahedron Lett.* **1980**, *21*, 3659-3662.

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