NARGENICIN BIOSYNTHESIS: LATE STAGE OXIDATIONS AND ABSOLUTE CONFIGURATION

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Nargenicin A_1 (1)[1] and the closely related antibiotic nodusmicin (2)[2], are members of a novel class of saturated alicyclic polyketides containing a characteristic *cis*-fused octalin ring system. A similar, *trans*-fused, octalin nucleus is found in the Actinomycete metabolites chlorothricin (3)[3], kijanimicin (4)[4], and tetrocarcin A (5)[5,6], as well as in the fungal antibiotic ilicicolin H (6)[7]. Related to the latter metabolite are the recently described fungal phytotoxins betaenones A (7) and B (8)[8] as well as the hypocholesterolemic agents compactin (9)[9] and mevinolin (10)[10]. We have recently described incorporation experiments which establish the acetate and propionate origin of the carbon skeleton of nargenicin[11]. Similar results were reported concurrently by RINEHART for the biosynthesis of nodusmicin[12]. We also reported that incorporation of [1-$^{18}O_2$, 1-^{13}C]acetate gave nargenicin which bore oxygen-18 at C-1 and C-11, as detected by the isotopically induced shifts of the corresponding ^{13}C NMR signals, while incorporation of [1-$^{18}O_2$, 1-^{13}C]propionate resulted in oxygen-18 enrichment at C-9 and C-17. These results have important implications for any proposed carbocyclization mechanism leading to the generation of the bicyclic octalin ring system from a reduced, linear polyketide precursor. The absence of propionate-derived oxygen at C-13 of nargenicin is inconsistent with a simple aldol-type condensation leading to the formation of the corresponding C-4, 13 bond, while the fact that neither the C-9 nor the C-11 oxygen atoms are derived from molecular oxygen rules out plausible epoxy-olefin or epoxy-alcohol cyclizations. As an alternative, we have suggested that the parent octalin ring system may be generated with the observed relative configuration and functional group distribution by an intramolecular Diels-Alder reaction between an *E,E*-4,6-diene and an *E* dienophile represented by the putative tetraene precursor (11). Similar mechanisms may in fact be advanced to account for the formation of related octalin-containing metabolites*. In continuation of these studies, we have now determined the biosynthetic origins of the remaining oxygen atoms of nargenicin and established for the first time the absolute configuration of the nargenicin-nodusmicin family of antibiotics.

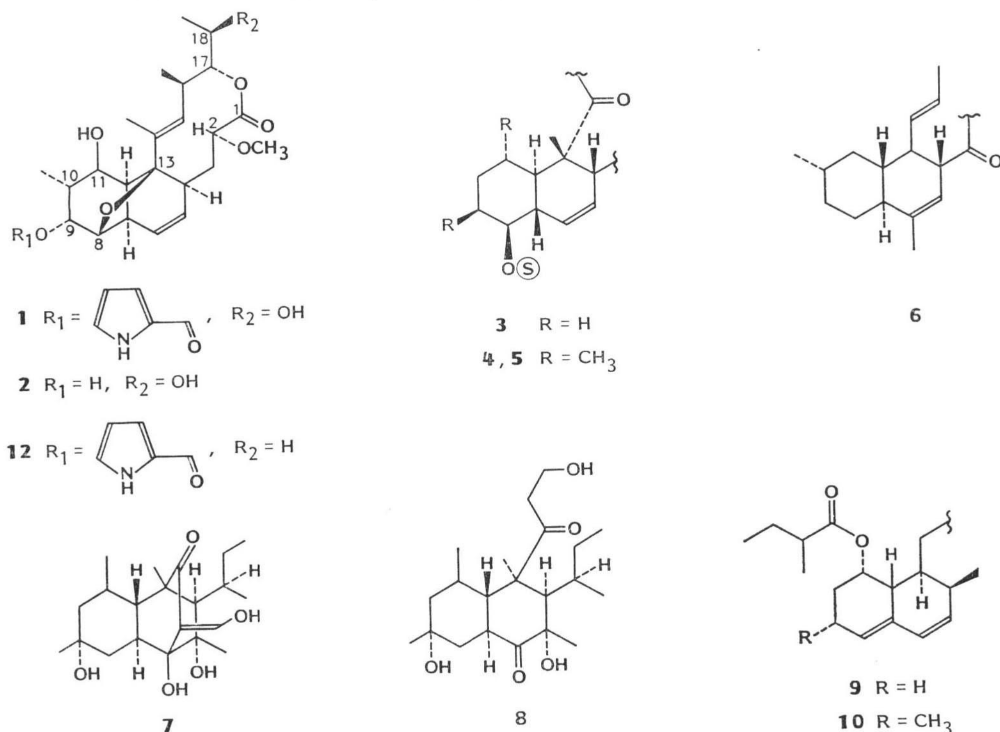
To determine the origin of the remaining oxygen atoms of nargenicin, *Nocardia argentinensis* Huang, ATCC 31306, was incubated in an atmosphere of ^{18}O-labeled molecular oxygen. Considerable preliminary experimentation was necessary in order to establish suitable fermentation and labeling conditions due the severe reduction in nargenicin production associated with reduced culture aeration. A 2,800-ml Fernbach flask containing 280 ml of the previously described fermentation medium[11] was inoculated with 100-ml of a vegetative culture of *N. argentinensis* and incubated at 30°C and 250 rpm for 24 hours. The culture was then flushed with nitrogen and connected to a closed system containing a peristaltic pump and manostat which was used to circulate 4 liters of a 3:1 mixture of N_2 and $^{18}O_2$ (97 atom %) directly through the fermentation medium at a flow rate of 50~60 ml/minute**. A series of three U-tubes containing, respectively, charcoal, 5 N HCl and 5 N NaOH, was used to trap evolved CO_2 and other volatile materials. In order to increase the intensity of the relevant ^{13}C NMR signals, and at the same time avoid unwanted excess intramolecular multiple ^{13}C labeling, the cultures were supplemented with a mixture of 0.15 g of sodium [1-^{13}C]propionate***, 0.15 g of sodium [2-^{13}C]propionate***, 0.54 g of unlabeled sodium propionate, 0.18 g of sodium [2-^{13}C]acetate***, 0.10 g of unlabeled sodium acetate, and 2.2×10^7 dpm of sodium [1-^{14}C]propionate at intervals of 24, 48 and 72 hours total incubation time in portions of 40%, 30% and 30%, respectively. After 96 hours, the resulting labeled nargenicin (10 mg) was isolated and purified as previously described[11]. Analysis by high resolution 62.9 MHz ^{13}C NMR revealed the pre-

* It is interesting to note that whereas the hypothetical *ring-forming* mechanism may be the same for nargenicin and for the *trans*-octalins 3, 4, and 5, the direction of polyketide *chain-elongation* is opposite for these two families of metabolites. See ref 15 and 16.

** For a description of this apparatus, see ref 17.

*** 90 Atom % ^{13}C.

Fig. 1. Structures of saturated bicyclic polyketides, nargenicin A_1 (**1**), nodusmicin (**2**), betaenone A (**7**), betaenone B (**8**) and 18-deoxynargenicin (**12**), and partial structures of chlorothricin (**3**), kijanimicin (**4**), tetrocarcin (**5**), ilicicolin H (**6**), compactin (**9**) and mevinolin (**10**).



Scheme 1. Proposed intramolecular Diels-Alder mechanism for biosynthesis of nargenicin and labeling of C-2, C-8,13, and C-18 oxygen atoms by molecular oxygen.

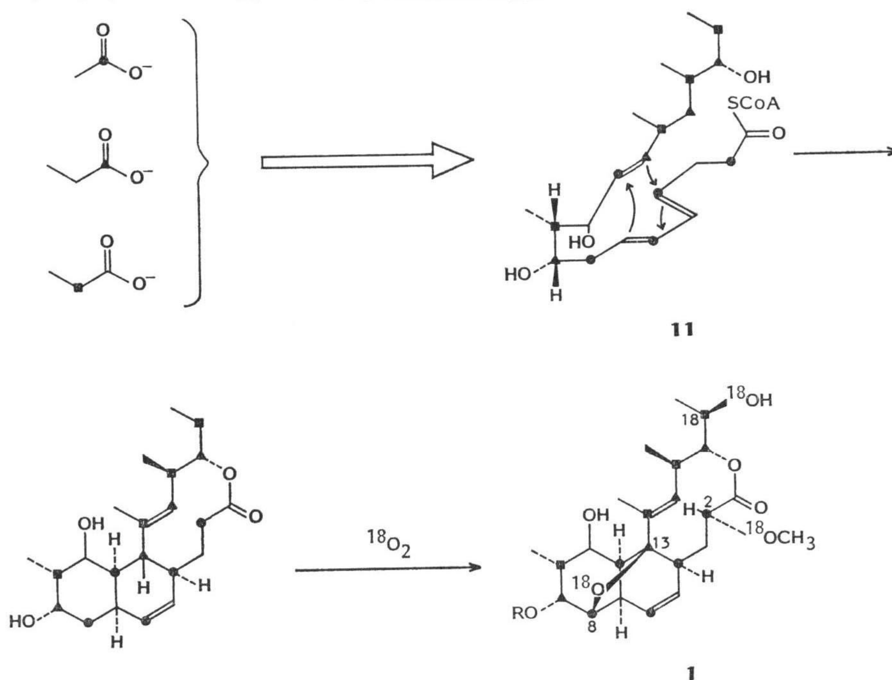


Table 1. Incorporation of $^{18}O_2$ into nargenicin A_1[a].

C	^{13}C shift (ppm)	Enrichment (%)	$\Delta\delta$ (ppm[b])	$^{18}O:^{16}O$[c]
2	83.4	4.9	0.018	35: 65
8	81.3	4.9	0.024	30: 70
13	89.1	3.0	0.030	30: 70
18	66.6	8.9	0.024	25: 75
23	57.6	1.6[d]	0.024	20: 80

a Bruker WM 250, 62.9 MHz; spectral width 12195 Hz; 64 K data points; quadrature detection; 60° pulse; repetition time 1.34 seconds, LB=0 GB=0; 0.010 g sample in 0.4 ml of $CDCl_3$.

b $^{13}C^{18}O$ isotope shift, ±0.006 ppm.

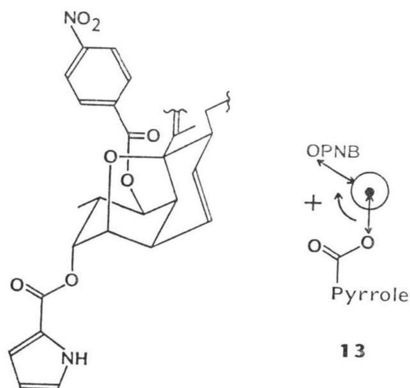
c ±5; uncorrected for contribution of natural abundance ^{13}C to $^{13}C^{18}O$ peaks.

d Indirect ^{13}C labeling.

sence of isotopically shifted signals corresponding to excess ^{18}O at C-2, C-8, C-13, C-18, and the methoxyl methyl, C-23 (Table 1). These results complement the earlier data from incorporation of labeled acetate and propionate and establish unambiguously that the two ether oxygen atoms of nargenicin, as well as the C-18 hydroxyl oxygen are derived, as previously predicted[11], from molecular oxygen. Although it is not possible to stipulate the order in which the various oxygen atoms are introduced, it is likely that oxidation at C-18 occurs last, based on the reported isolation of 18-deoxynargenicin A_1 (**12**) from *Saccharopolyspora hirsuta*, the organism which produces nodusmicin[13]. To date, no further deoxygenated nargenicin or nodusmicin derivatives have been isolated and the detailed mechanism of formation of the C-8,13 ether bridge remains obscure.

While the structure and relative stereochemistry of nargenicin A_1 and nodusmicin have been unambiguously assigned by detailed ^1H NMR and X-ray crystallographic analyses[1,2], as well as by chemical conversion of **2** to **1**, to date the absolute configuration of these two antibiotics has not been reported. To solve this problem, we chose the nonempirical CD exciton chirality method of HARADA and NAKANISHI[14]. The ether-bridged, *cis*-fused octalin system provides a suitable rigid template bearing one of the two necessary chromophores, the pyrrole carboxylate moiety (λ_{max}^{MeOH} 262 nm (ε 12,441)) which is esterified to the axial C-9α hydroxyl group. Selective acetylation (1 equiv Ac_2O, pyridine, 25°C, 18 hours) of the side chain (C-18) hydroxyl function, followed by *p*-nitrobenzoylation (*p*-nitrobenzoyl chloride, pyridine, catalytic 4-*N*,*N*-dimethylaminopyridine, reflux, 12 hours; λ_{max} 252 nm) of the equatorial C-11β hydroxyl group, gave the corresponding 11-*p*-nitrobenzoyl, 18-acetylnargenicin (**13**), λ_{max}^{MeOH} 260.5 (ε 15,036). The CD spectrum of **13** in MeOH showed a strong positive Cotton effect: $\Delta\varepsilon$ (273.3 nm) +8.9, $\Delta\varepsilon$ (252.3 nm) −2.4, $\Delta\Delta\varepsilon$ 11.1. The positive Cotton effect corresponds to a clockwise relationship between the pyrrole carboxylate and *p*-nitrobenzoate chromophores[14], as illustrated in Fig. 2. Nargenicin A_1 is therefore shown to have the absolute configuration represented by **1***.

Fig. 2. Partial structure of 11-*p*-nitrobenzoyl, 18-acetylnargenicin (**13**), showing clockwise relationship of pyrrole carboxylate and *p*-nitrobenzoate (PNB) chromophores.



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* The relative stereochemical assignments are based on the X-ray structure in Fig. 1 of ref 2. The usual way of drawing the configuration of the C-2 methoxyl group is ambiguous and does, in fact, tend to suggest the wrong relative stereochemistry at the latter center. Note also that the configuration at C-18 is incorrectly drawn in ref 11.

References

1) CELMER, W. D.; G. N. CHMURNY, C. E. MOPPETT, R. S. WARE, P. C. WATTS & E. B. WHIPPLE: Structure of natural antibiotic CP-47,444. J. Am. Chem. Soc. 102: 4203~4209, 1980
2) WHALEY, H. A.; C. G. CHIDESTER, S. A. MIZSAK & R. J. WNUK: Nodusmicin: The structure of a new antibiotic. Tetrahedron Lett. 21: 3659~3662, 1980
3) MUNTWYLER, R. & W. KELLER-SCHIERLEIN: Metabolic products of microorganisms. 107. Structure of chlorothricin, a new type of macrolide antibiotic. Helv. Chim. Acta 55: 2071~2094, 1972
4) MALLAMS, A. K.; M. S. PUAR, R. R. ROSSMAN, A. T. MCPHAIL, R. D. MACFARLANE & R. L. STEPHENS: Kijanimicin. 3. Structure and absolute stereochemistry of kijanimicin. J. Chem. Soc. Perkin Trans. I 1983: 1497~1534, 1983
5) TOMITA, F.; T. TAMAOKI, K. SHIRAHATA, M. KASAI, M. MORIMOTO, S. OHKUBO, K. MINEURA & S. ISHII: Novel antitumor antibiotics, tetrocarcins. J. Antibiotics 33: 668~670, 1980
6) HIRAYAMA, N.; M. KASAI, K. SHIRAHATA, Y. OHASHI & Y. SASADA: The structure of tetronolide, the aglycone of antitumor antibiotic tetrocarcin. Tetrahedron Lett. 21: 2559~2560, 1980
7) MATSUMOTO, M. & H. MINATO: Structure of ilicicolin H, an antifungal antibiotic. Tetrahedron Lett. 1976: 3827~3830, 1976
8) ICHIHARA, A.; H. OIKAWA, K. HAYASHI, S. SAKAMURA, A. FURUSAKI & T. MATSUMOTO: Structures of betaenones A and B, novel phytotoxins from *Phoma betae* Fr. J. Am. Chem. Soc. 105: 2907~2908, 1983
9) BROWN, A. G.; T. C. SMALE, T. J. KING, R. HASENKAMP & R. H. THOMPSON: Crystal and molecular structure of compactin, a new antifungal metabolite from *Penicillium brevicompactum*. J. Chem. Soc. Perkin Trans. 1976: 1165~1170, 1976
10) ENDO, A.: Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. J. Antibiotics 32: 852~854, 1979
11) CANE, D. E. & C.-C. YANG: Biosynthetic origin of the carbon skeleton and oxygen atoms of nargenicin A_1. J. Am. Chem. Soc. 106: 784~787, 1984
12) SNYDER, W. C. & K. L. RINEHART, Jr.: Biosynthesis of nargenicin and nodusmicin. J. Am. Chem. Soc. 106: 787~789, 1984
13) WHALEY, H. A. & J. H. COATS: Nodusmicin and related antibiotics from a *Saccharopolyspora* fermentation. Abstracts of Papers Intersci. Conf. Antimicrob. Agents Chemother., No. 187, Chicago, IL, 1981
14) HARADA, N. & K. NAKANISHI: Circular Dichroic Spectroscopy. Exciton Coupling in Organic Stereochemistry. University Science Books, Mill Valley, CA, 1983
15) MASCARETTI, O. A.; C.-J. CHANG, D. HOOK, H. OTSUKA, E. F. KREUTZER & H. G. FLOSS: Biosynthesis of the macrolide antibiotic chlorothricin. Biochemistry 20: 919~924, 1981
16) TAMAOKI, T. & F. TOMITA: Biosynthesis of tetrocarcin. Incorporation of ^{14}C- and ^{13}C-labeled compounds into tetrocarcin. J. Antibiotics 36: 595~598, 1983
17) CANE, D. E.; T.-C. LIANG & H. HASLER: Polyether biosynthesis. 2. Origin of the oxygen atoms of monensin A. J. Am. Chem. Soc. 104: 7274~7281, 1982