Biosynthesis of Nigrifactin

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Summary In the biosynthesis of nigrifactin (**1**), produced by *Streptomyces*, (**1**) is derived from six acetate units in a linear combination; 5-oxododecanal (**3**), 2-n-heptyl-3,4,5,6-tetrahydropyridine, (**4**), and 2-n-heptylpiperidine (**5**) are probable intermediates.

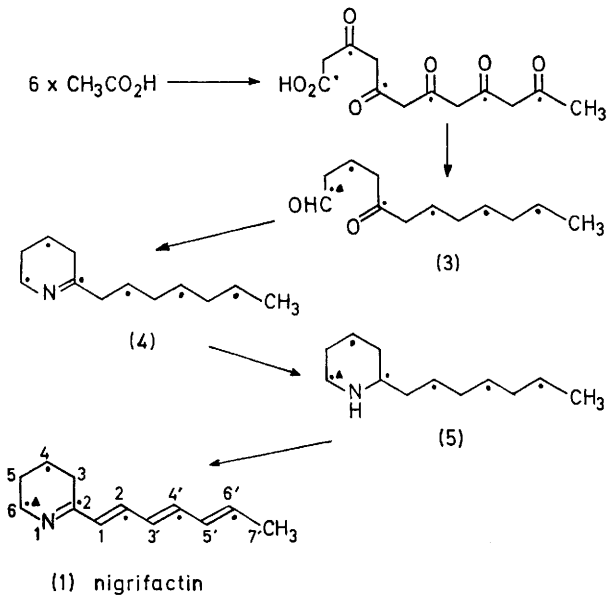
THE biosynthesis of simple piperidine alkaloids is well studied; the piperidine ring can be derived from lysine, acetic acid, or terpenoids.[1] Recently, several simple piperidine alkaloids have been isolated from micro-organisms,[2–4] but their biosynthesis has not been examined. We have studied the biosynthesis of nigrifactin (**1**)[3] produced by *Streptomyces*.

Isotope-enriched precursor was added to the culture medium[3] of *Streptomyces nigrifaciens* var. FFD-101 at the most active period of nigrifactin biosynthesis. Fermenta-

TABLE

Compound fed to *Streptomyces*	Abs. incorpn. (%)	Distribution[c] (%) of activity C-6	C-2	C-1′—C-7′
[1-^{14}C]Acetic acid[a]	6—8	17	20	48
[2-^{14}C]Acetic acid[a]	6—8	*ca.* 3	—	—
[6-^{14}C]DL-Lysine[b]	0—0·1	*ca.* 8	—	—
[1-^{14}C]-5-Oxododecanoic acid (**2**)[b,c] ..	1·1[d]	17	—	—
[1-^{14}C]-5-Oxododecanal (**3**)[b,c]	1·4[d]	96[e]	—	—
[6-^{14}C]-2-n-Heptylpiperideine (**4**)[b,c] ..	0·05[d]	100	—	—
[6-^{14}C]-2-n-Heptylpiperidine (**5**)[b,c] ..	0·1[d]	100	—	—

[a] Fermentation terminated 12 h after addition of precursor. [b] Fermentation terminated 1 h after addition of precursor. [c] The synthesis of these compounds and the degradation of (**1**) to determine the location of the activity will be reported in due course. [d] Although approximately equal amounts of the precursors were fed to *Streptomyces*, comparison of the incorporation rate between these precursors may not have much meaning, because the solubility of these materials in the culture medium is very poor and also the efficiency of the permeability of these materials through the cell wall is open to question. [e] 5-Oxododecanal (**3**) is quite labile to oxygen and is contaminated with 5-oxododecanoic acid (**2**). This might make the isotope distribution at C-6 slightly lower (4%) than expected.



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tion was terminated 1 or 12 h after the addition of the precursor and the isotope-enriched nigrifactin picrate (**1**) (m.p. 175—176 °C) was isolated by Kaneko's method.[3]

As expected, nigrifactin (**1**) gives twelve signals in the natural-abundance proton-noise-decoupled ^{13}C n.m.r. spectrum.† The enhancement of the 6-, 4-, 2-, 2′-, 4′-, and 6′-signals† in the spectrum of a sample enriched biologically with [1-^{13}C]acetic acid shows clearly that the carbon atoms at these positions in (**1**) are derived from the carboxy-group of acetic acid,‡ and suggests that nigrifactin (**1**) is biosynthesized from six acetate units in a linear combination like hemlock alkaloids.[1,5] This conclusion is also supported by ^{14}C tracer experiments (see Table).

To study the biosynthetic intermediates between acetate and (**1**), several probable compounds,[1] specifically labelled with ^{14}C, were synthesized and fed to *Streptomyces* and fermented for 1 h. The results are summarized in the Table. Compounds (**3**), (**4**), and (**5**) were assumed to be the intermediates, in view of the location of the activity. The fact that (**5**) is an efficient precursor suggests that the double bonds in (**1**) are introduced in the last step.

A possible biosynthetic pathway to nigrifactin (**1**) is in the Scheme.

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† Detailed data on the assignment will be reported in due course. Off-resonance experiments are consistent with the assignment.

‡ Further confirmed by the ^{13}C n.m.r. spectra of octahydronigrifactin (**5**).

[1] E. Leete, *Accounts Chem. Res.*, 1971, **4**, 100, and references cited therein.
[2] A. I. Gurevich, M. N. Kolosov, V. G. Korobko, and V. V. Onopreinko, *Tetrahedron Letters*, 1968, 2209.
[3] T. Terashima, Y. Kuroda and Y. Kaneko, *Tetrahedron Letters*, 1969, 2535; *Agric. and Biol. Chem.* (*Japan*), 1970, **34**, 747, and references cited therein.
[4] S. Umezawa, K. Tatsuta, Y. Horiuchi, and T. Tsuchiya, *J. Antibiotics*, 1970, **23**, 28.
[5] This pathway has been predicted by Leete (see ref. 1).