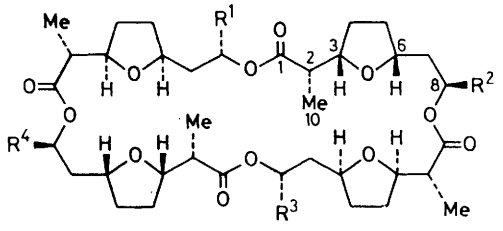
Biosynthesis of Nonactin from Acetate, Propionate, and Succinate; the Assignment of its Carbon-13 N.M.R. Spectrum by Two-dimensional Correlation Spectroscopy

Doreen M. Ashworth, John A. Robinson, and David L. Turner

Department of Chemistry, University of Southampton, Southampton SO9 5NH, U.K.

Two-dimensional correlation spectroscopy is used to assign unambiguously the carbon-13 n.m.r. spectrum of nonactin, and studies with ^{13}C labelled acetate, propionate, and succinate have prompted a revision of its mode of biosynthesis in *Streptomyces griseus*.

The macrotetrolides (**1**)—(**5**) are ionophore antibiotics produced by several strains of Streptomyces.[1] The macrocyclic ring of nonactin (**1**), the parent compound, is very unusual in that it is a tetramer composed of both enantiomers of nonactic acid and these are combined in a (+)(−)(+)(−) fashion with the result that nonactin is an achiral molecule possessing S_4 symmetry. An assignment of the $^{13}C-\{^1H\}$ n.m.r. spectrum of nonactin made with the aid of additivity rules, chemical shift reagents, and spectra of model compounds has been reported,[2] but as a prelude to a detailed biosynthetic investigation we have confirmed this assignment unambiguously from a two-dimensional carbon-13 autocorrelation experiment using the natural abundance carbon-13 satellites of the $^{13}C-\{^1H\}$ spectrum.[3] The double quantum transition of the coupled spins



(**1**), Nonactin; $R^1 = R^2 = R^3 = R^4 = Me$
(**2**), Monactin; $R^2 = Et$, $R^1 = R^3 = R^4 = Me$
(**3**), Dinactin; $R^2 = R^4 = Et$, $R^1 = R^3 = Me$
(**4**), Trinactin; $R^2 = R^3 = R^4 = Et$, $R^1 = Me$
(**5**), Tetranactin; $R^1 = R^2 = R^3 = R^4 = Et$

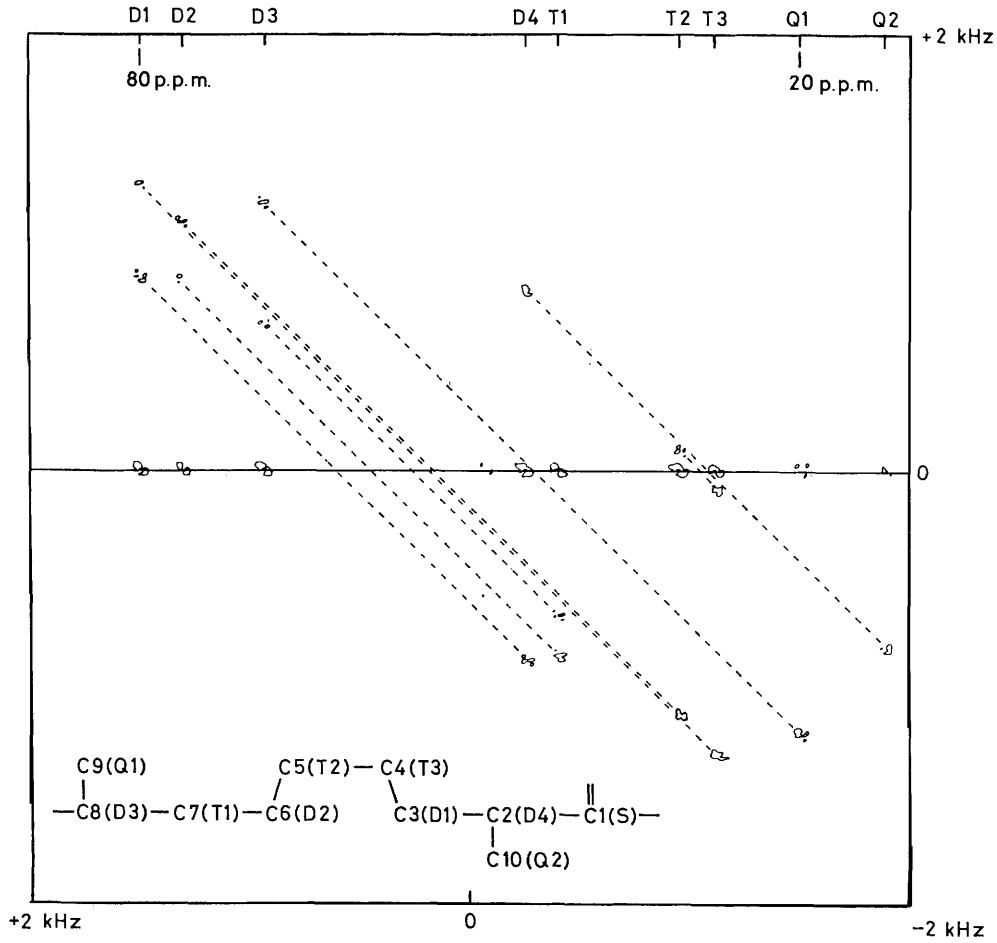


Figure 1. Part of the autocorrelation spectrum of nonactin; obtained in 15 h from *ca.* 1 g of nonactin dissolved in 2 ml of $CDCl_3$ using a Bruker CXP200 spectrometer operating at 50.3 MHz. Proton coupled multiplet structure for each resonance is indicated by D = doublet, T = triplet, and Q = quartet. Correlated nuclei are indicated by dotted lines. The carbon connectivity pattern, apart from the bond to C(1) whose resonance is not shown, is clearly discernible.

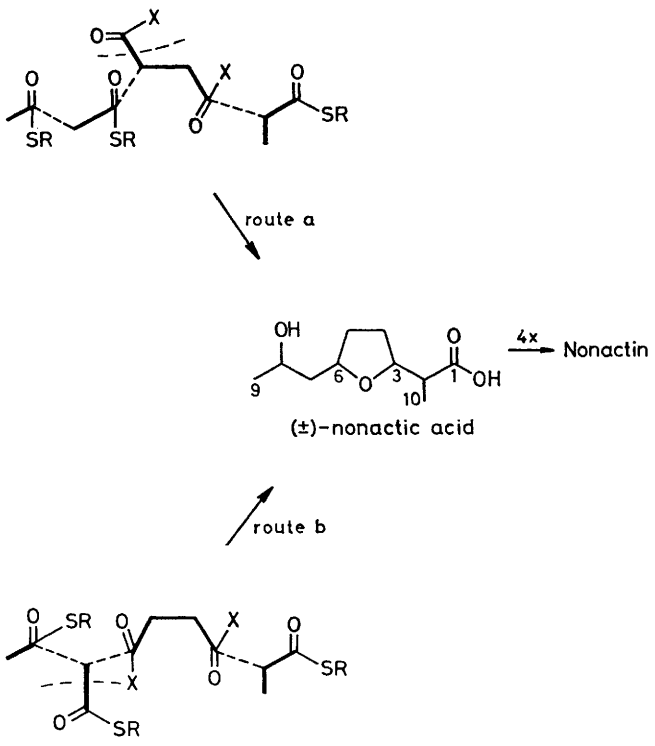
in molecules containing contiguous pairs of carbon-13 nuclei is used to suppress the intense singlets from isolated carbon-13 nuclei[4] and leave only doublets with splittings of the order of 40 Hz, a typical one-bond coupling constant. The autocorrelation spectrum shown in Figure 1 was obtained using method B of reference 3, in which responses from correlated signals appear in the second (vertical) dimension at half the difference of the chemical shifts which are shown horizontally. Since the lines in this spectrum are well dispersed other methods of assignment based on the repeated doublet splittings of AX or AB groups[5] might be sufficient if the coupling constants vary enough, but such methods are totally inadequate for more complex molecules.[6]

The biosynthesis of nonactin has been studied previously[7] using ^{14}C labelled precursors, as well as [1,4-$^{4}C_2$, 2-^{3}H] succinate, and these investigations indicated that route a in Scheme 1 is used by *Streptomyces griseus* for nonactin production. The results of ^{13}C-labelling experiments with the precursors shown in Table 1 are, however, inconsistent with this approach, but support route b shown in Scheme 1. A fer-

Table 1. Enrichments of carbon-13 in nonactin from ^{13}C labelled acetate, propionate, and succinate.

	% Enrichment[b] of ^{13}C in nonactin									
Precursor added[a]	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
[1-^{13}C] acetate	2.6	—	4.9	—	—	2.6	—	6.6	—	—
[2-^{13}C] acetate	1.5	3.1	2.1	4.2	4.1	2.1	5.5	—	7.4	3.9
[1,4-$^{13}C_2$] succinate	2.9	—	3.1	—	—	3.2	—	—	—	—
[1-^{13}C] propionate	1.9	—	—	—	—	—	—	—	—	—
	Coupling constant of enriched ^{13}C–^{13}C doublets/Hz									
[1,2-$^{13}C_2$] acetate	—	—	35	35	34	34	—	39	39	—
[2,3-$^{13}C_2$] succinate	—	34	—	32	32	—	—	—	—	34

[a] Each site 90 atom % enriched; doubly labelled precursors were diluted at least 1 : 1 with unlabelled material. [b] Enrichment = (relative height of carbon-13 resonance in labelled nonactin)/(relative height of same resonance at natural abundance), measured under identical spectrometer conditions.



Scheme 1. X = SR, OH.

mentation of *S. griseus* strain ETH A7796 (100 ml) was supplemented with each of the labelled molecules shown in Table 1 so that their final concentration was 20 mM. Six days after inoculation and one to two days after addition of labelled compound, the nonactin was isolated, purified, and examined by ^{13}C n.m.r. at 25 MHz. The incorporation of ^{13}C label to equal extents into C(3) and C(6) of nonactin biosynthesized from [1,4 $^{13}C_2$] succinate, together with the incorporation of intact C_2 units from [1,2-$^{13}C_2$] acetate into C(8)–C(9), C(3)–C(4), and C(5)–C(6), but not C(6)–C(7), are inconsistent with route a, but support route b. The observed incorporations of ^{13}C label from the acetate into the tetrahydrofuran ring of nonactic acid are explained by the operation of a citric acid cycle which converts acetyl–CoA, derived from the administered acetate, into succinyl–CoA and succinate. The efficient incorporation of label from [1,4-$^{13}C_2$] succinate into C(1) of nonactic acid, probably arises through the conversion of succinyl–CoA into methylmalonyl–CoA by an active methylmalonyl–CoA mutase, as does the C(2)–C(10) coupling observed from the [2,3-$^{13}C_2$] succinate experiment. The administration of [1-^{13}C]propionate stimulates the production of monactin (**2**) enriched at C(8), as well as nonactin labelled at C(1).

The previously observed[7] increase in $^{3}H/^{14}C$ ratio upon incorporation of [1,4-$^{14}C_2$, 2-^{3}H] succinate into the tetrahydrofuran ring of nonactic acid, which appears to support route a, can be rendered compatible with route b by assuming that the added precursor is metabolized *via* the citric acid cycle faster than its direct incorporation into nonactic acid. An intermolecular tritium kinetic isotope effect operative during the succinate dehydrogenase reaction could then lead to a substantial enrichment of tritium in succinate prior to incorporation.

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