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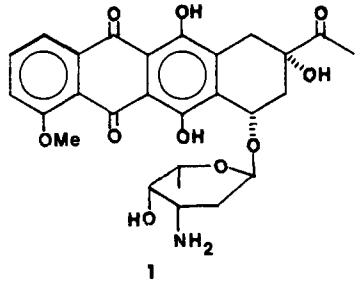
Communications to the Editor

A ^{13}C Nuclear Magnetic Resonance Study of the Biosynthesis of Daunomycin from $^{13}CH_3{}^{13}CO_2Na$

Sir:

A number of plausible connectivity patterns are indicated (Scheme I) for the biosynthesis of the polyketide antibiotic daunomycin (**1**) via an acetate–polymalonate route (Scheme I-c or d) or a propionate–polymalonate pathway (Scheme Ia or b). We have applied the Tanabe technique[1] to this biosynthetic problem and have obtained results that are consistent with only route a: a propionate "starter" and nine successive malonate condensations with loss of the terminal carboxyl.

Streptomyces peucetius (ATCC no. 21354) was maintained on malt extract agar[2a] and grown in a dry yeast/glucose production medium under submerged conditions in shake flasks. After considerable experimentation yields of **1** on the order of



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15 μg/ml could be isolated. For carbon NMR (^{13}C NMR) studies crude **1** was subjected to hydrolysis and acetylation with formation of daunomycinone tetracetate (**2**).

Through the use of the $Cr(acac)_3$ T_1 suppresor technique[3,4] peaks for all carbons are observed and are of comparable intensity in the ^{13}C NMR spectrum of **2** (Figure 1a). Application of chemical shift data from comparable anthraquinones,[3] substituent shift calculations,[5] and single frequency and off-resonance decoupling experiments allowed, with one or two ambiguities in the 135 ppm region, assignment of all peaks. These assignments are presented in Table I.

Scheme I

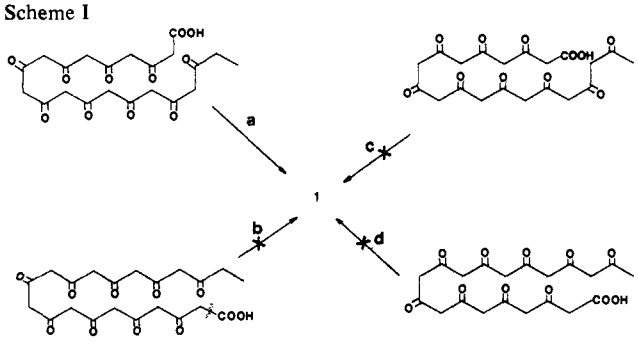
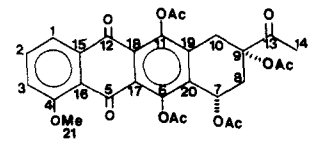


Table I. Chemical Shift Assignments for Carbons of Daunomycinone Tetraacetate (tabulates by the numbering system shown).



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Carbon	Chemical shift[a]	Carbon	Chemical shift
C-1	119.3[b]	C-13	205.0
C-2	134.1[b]	C-14[b]	24.0
C-3	118.3[b]	C-15	135.8
C-4	159.7	C-16[h]	122.3
C-5	180.6[d]	C-17[c]	126.2
C-6	146.7[c]	C-18[c]	125.2
C-7	62.1[b]	C-19[c]	134.7
C-8	30.9[b]	C-20[c]	134.2
C-9	80.4[e]	C-21[b]	57.0
C-19	31.3[b]	acetate methyls	20.8–21.0
C-11	145.1[c]	acetate carbonyls	168.9–170.7
C-12	182.0[d]		

[a] Chemical shifts were measured in $CDCl_3$ in parts per million relative to internal Me_4Si. [b] This carbon was positively assigned on the basis of single frequency decoupling experiments. [c] The C-11, C-6; C-17, C-18; and C-19, C-20 pairs were distinguished primarily by the single labeled acetate experiments. [d] C-5 and C-12 were distinguished by the single-labeled acetate experiments, but a comparison of this compound with islandicin triacetate would predict the given chemical shifts. [e] Sharp singlet in off-resonance decoupling experiments.

The incorporation experiments with ^{13}C-labeled sodium acetate (C_1, C_2, $C_{1,2}$ all 91% isotopic purity) entailed culturing *S. peucetius* in the dry yeast/glucose medium for 5 days and pulsing the growing organism twice daily on the second, third, and fourth days with 50 mg of the labeled acetate. The results of these experiments are presented in Table II, summarized in Figure 2, and discussed below.

CH_3 $^{13}CO_2Na$: Growth of *S. peucetius* as above in the presence of sodium [1-^{13}C]acetate afforded **2**, the ^{13}C NMR spectrum of which showed appreciable incorporation of ^{13}C at carbons 2, 4, 5, 6, 7, 15, 18, and 19.

Table II. The Enrichment Levels at the Various Carbons of Daunomycinone Tetraacetate from Sodium [1-^{13}C]- and [2-^{13}C] Acetate

Carbon	Peak intensity[a] Natural abundance	From [1-^{13}C] NaOAc	From [2-^{13}C] NaOAc
C-1	1.68	1.00	3.89
C-2	1.07	2.31	1.02
C-3	0.97	0.76	3.10
C-4	1.21	1.84	0.68
C-5	1.21	1.71	1.02
C-6	1.39	1.53	0.91
C-7	2.21	3.25	1.70
C-8	2.54	1.12	5.61
C-9	2.60	1.04	2.00
C-10	2.57	1.31	6.20
C-11	1.43	0.86	2.96
C-12	1.43	0.71	2.87
C-13	1.07	0.55	1.04
C-14	1.25	0.73	2.28
C-15	1.50	1.98	0.75
C-16	1.36	0.90	2.82
C-17	1.50	0.64	2.60
C-18	1.68	1.84	0.84
C-19	1.82	1.84	1.04
C-20	1.32	0.51	2.34
C-21	1.00	1.00	1.00

[a] Peak intensities were standardized to the methoxyl carbon.

$^{13}CH_3CO_2Na$: The pulsing of *S. peucetius* cultures with sodium [2-^{13}C]acetate resulted in the enhancement of carbons 1, 3, 8, 10, 11, 12, 16, 17, and 20.

It was clear from these two results that the biosynthesis of daunomycin does not conform to a "classical" acetate–polymalonate pathway (Scheme Ic or d). In particular the carboxyl and methyl derived carbons are opposite from those predicted by these two routes; the three-carbon fragment, carbons 9, 13, and 14, is not acetate derived at all, and there is one more methyl derived carbon than there are carboxyl-derived carbons.

$^{13}CH_3{}^{13}CO_2Na$: The ^{13}C NMR spectrum of **2** derived from this labeled acetate (Figure 1b) permitted the following strong conclusions in conjunction with the above results. (1) The three-carbon fragment [9, 13, 14] is either not acetate derived or is derived by a process that entails both dilution of the isotope (relative to other carbons in the molecule) and loss of the integrity of the added two-carbon acetate fragments.

(2) Whereas carbons 7 and 8 appear to be derived from the same acetate unit, carbon 10 is a singlet. This conclusion uniquely requires path a (Scheme I). Although this conclusion could be reversed (path b) if the assignments of C-8 and C-10 were reversed and C-19 and C-10, and C-7 and C-20 were coupled, this seems unlikely on the basis of single frequency decoupling experiments (C-8 vs. C-10) and other pairings that also implicate path a (see below).

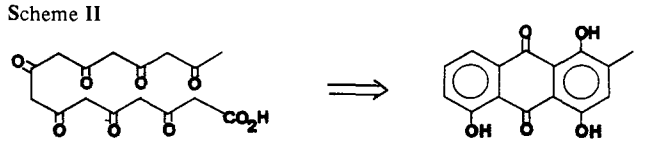
(3) Carbons 5 and 17 appear to be derived from the same acetate unit as do carbons 4 and 16.

(4) Carbon 3 appears with a satellite doublet (J = 68 Hz) and is presumably coupled to C-2. The splitting of C-2, immersed in the 135 ppm complex, could not be determined. The distinction between C-1 and C-3 rests on single frequency decoupling experiments, C-3 being coupled to the higher field aromatic hydrogen. Even if this assignment were reversed C-3 cannot be coupled to C-4; it must be coupled to C-2.

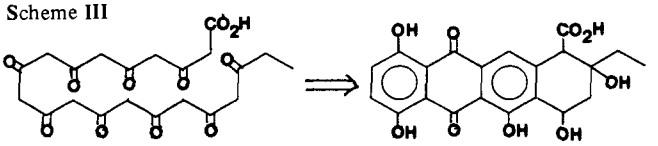
(5) Both C-12 and C-18 have satellite doublets (J = 55 Hz); these can be derived from the same acetate unit. In particular C-18 is not coupled to C-11. Although C-18 could be coupled to C-17, this would require a most esoteric connectivity pattern.

The above results are uniquely consistent with pathway a and show that the acetate connectivity pattern is both strikingly different from that determined for the similar polyketide islandicin[3] (Scheme II) and strikingly similar to that proposed some time ago for the metabolite rutilantinone[6] (Scheme III), wherein the three-carbon fragment corresponding to carbons 19, 13, and 14 was shown to be propionate derived.[6,7]

Scheme II



Scheme III



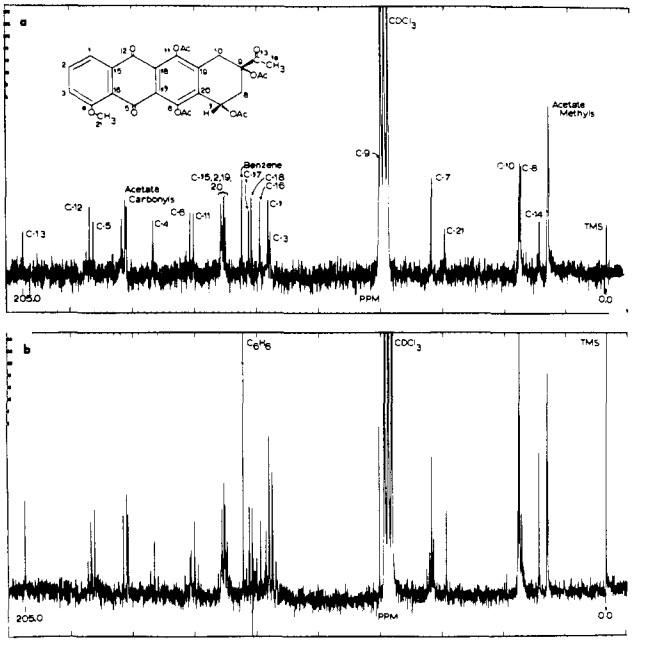


Figure 1.

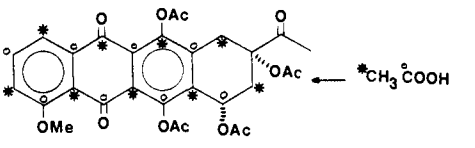


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Figure 2. The labeling pattern established by the incorporation of sodium [1-^{13}C]- and [2-^{13}C]acetate into daunomycin.

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