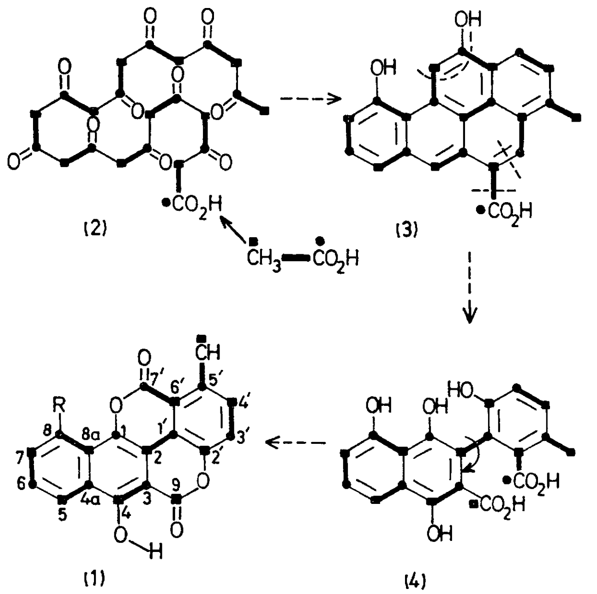
Pattern of Acetate Incorporation into the Aglycone of Chartreusin. Evidence from ^{13}C Nuclear Magnetic Resonance Studies for a Single-chain Polyketide Intermediate

By Phyllis Canham and Leo C. Vining*
(*Department of Biology, Dalhousie University, Halifax, Nova Scotia,* B3H 4J1, *Canada*)
and A. Gavin McInnes, John A. Walter, and Jeffrey L. C. Wright
(*Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia,* B3H 3Z1, *Canada*)

Summary ^{13}C N.m.r. analysis of the labelling pattern in chartreusin biosynthesized from singly and doubly ^{13}C-labelled acetate has indicated that the aglycone is formed by condensation and subsequent scission of a single polyketide chain of 22 carbon atoms.

Chartreusin (**1**: R = 2-*O*-α-D-digitalopyranosyl-β-D-fucopyranoside), the isocoumarin glycoside antibitoic produced by *Streptomyces chartreusis*[1] is known to be derived, in part, from acetate.[2,3] Brown and his co-workers[2] interpreted the labelling pattern in the aglycone after incorporation of ^{14}C-labelled acetate and malonate as evidence for its biosynthesis *via* angular or linear tetracyclic compounds, or from two separate polyketide chains. The information from such studies was indecisive but the progenitor was assumed to derive from ten acetate units. We have re-examined this question using [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C]-acetate as precursors and ^{13}C n.m.r. spectroscopy to elucidate not only the biogenetic origin of each aglycone carbon but also the pattern of bonded two-carbon unit incorporation.

Aglycone resonances in the ^1H coherent broad-band-decoupled[4] pulse Fourier transform ^{13}C n.m.r. spectrum of (**1**) in $(CD_3)_2SO$ were assigned from correlations between chemical shifts relative to internal Me_4Si, direct and long-range spin–spin couplings to hydrogen measured from high-resolution spectra, and unambiguous pairing of carbon atoms that were spin–spin coupled owing to incorporation of intact [1,2-^{13}C]acetate ($\overset{\blacksquare}{C}H_3\text{–}\overset{\bullet}{C}O_2H$). Assignments were: δ 163·6 (C-9), 158·3 (C-7′), 155·4 (C-4), 154·0 (C-8), 145·8 (C-2′), 138·5 (C-5′), 138·4 (C-1), 133·1 (C-4′, $^1J_{CH}$ 164·4 Hz), 128·3 (C-6, $^1J_{CH}$ 165·0 Hz), 126·0 (C-4a), 120·6 (C-3′, $^1J_{CH}$ 166·3 Hz), 119·2 (C-1′), 117·8 (C-8a), 116·7 (C-6′), 116·2 (C-5, $^1J_{CH}$ 167·8 Hz), 114·4 (C-7, $^1J_{CH}$ 162 ± 2 Hz), 108·1 (C-2), 96·5 (C-3), and 21·6 (CH_3, $^1J_{CH}$ 126·7 Hz). Chartreusin from cultures supplemented with 90% enriched [1-^{13}C]-acetate (●) showed enhanced signal intensity (*ca.* 3% ^{13}C above natural abundance) for C-1, C-3, C-4a, C-6, C-8, C-1′, C-3′, C-5′, and C-7′. The remaining carbon atoms of the aglycone were labelled to the same extent by [2-^{13}C]acetate (■), thus confirming the labelling pattern proposed by Schmid.[3] Coupled pairs of carbon atoms in the spectrum of (**1**) labelled with [1,2-^{13}C]acetate (^{13}C enrichment *ca.* 0·5%) were found by matching ^{13}C–^{13}C satellite spacings, and confirmed by homonuclear single-frequency ^{13}C decoupling[5] or changes in $^1J_{CC}$ values resulting from off-resonance effects, as follows: $^1J_{CC}$ C-3, C-4, 70·6; C-4a, C-5, 56·5; C-6, C-7, 54·7 ± 0·8; C-8, C-8a, 68·4; C-2, C-1′, 53·9; C-3′, C-4′, 54·5; Me, C-5′, 43·5; C-6′, C-7′, 74·1 Hz.



Scheme

Although all 19 carbon atoms of the aglycone are derived from acetate, only eight intact two-carbon units are present. C-2′ and C-9 originate from the methyl and C-1 from the carboxy-group by C–C bond scission. From the arrangement of ^{13}C–^{13}C units and location of the three uncoupled carbon atoms it is apparent that 11 acetate units participate in the formation of (**1**). A plausible first step would be an acetate–polymalonate condensation to give the single 22 carbon atom polyketide chain (**2**) which, by cyclization to the benzpyrene-like intermediate (**3**) and subsequent bond cleavage, would lose three carbon atoms to give (**4**). Rotation of the phenyl component followed by lactone formation would yield (**1**) with the correct disposition of labels (Scheme). Alternative biogenetic schemes invoking three or more polyketide chains are possible, but are considered less likely.

Chartreusin aglycone is unusual among polyketide secondary metabolites in being derived from 11 acetate units.[6] The evidence that this large molecule originates from a single polyketide chain suggests that similar biogeneses might be entertained for other acetate-derived compounds now thought to be of mixed polyketide origin.

(*Received*, 17*th February* 1976, *Com.* 169.)

---

[1] B. E. Leach, K. M. Calhoun, L. E. Johnson, and W. G. Jackson, *J. Amer. Chem. Soc.*, 1953, **75**, 4011; J. Berger, L. H. Sternbach, R. G. Pollock, E. R. LaSala, S. Kaiser, and M. W. Goldberg, *ibid.*, 1958, **80**, 1636; E. Simonitch, W. Eisenhuth, O. A. Stamm, and H. Schmid, *Helv. Chim. Acta*, 1960, **43**, 58; W. Eisenhuth, O. A. Stamm, and H. Schmid, *ibid.*, 1964, **47**, 1477.

[2] J. R. Brown, M. S. Spring, and J. R. Stoker, *Phytochemistry*, 1971, **10**, 2056.

[3] H. Schmid, *Angew. Chem.*, 1963, **75**, 347.

[4] J. B. Grutzner and R. E. Santini, *J. Magnetic Resonance*, 1975, **19**, 173.

[5] A. G. McInnes, D. G. Smith, J. A. Walter, L. C. Vining, and J. L. C. Wright, *J.C.S. Chem. Comm.*, 1975, 66.

[6] W. G. Turner, 'Fungal Metabolites,' Academic Press, London, 1971, p. 191.