Biosynthesis of the Diterpene Antibiotic, Aphidicolin, by Radioisotope and ^{13}C Nuclear Magnetic Resonance Methods

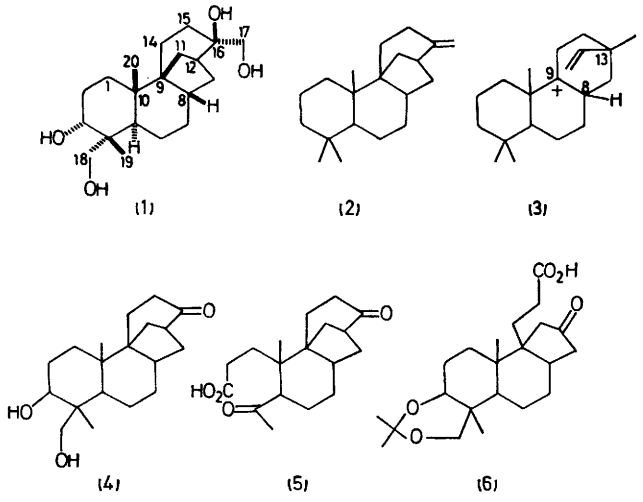
By MARTIN R. ADAMS and JOHN D. BU'LOCK*

(*Weizmann Microbial Chemistry Laboratory, Department of Chemistry, The University, Manchester* M13 9PL)

Summary Spectroscopic and degradative methods with 1,2-$^{13}C_2$-acetate and 2-^{14}C-4*R*-^{3}H-mevalonate precursors show that aphidicolin arises from initial chair-boat cyclization of the diterpene chain followed by successive H-shift, cyclization, and rearrangement steps.

APHIDICOLIN (**1**) is an antiviral antibiotic produced by the fungi *Cephalosporium aphidicola*[1] and *Nigrospora sphaerica*,[2,3] whose structure has the absolute stereochemistry shown.[4] It is probably formed by oxygenation of aphidicolene (**2**), one of several co-occurring diterpene hydrocarbons in both organisms.[3,4] As pointed out by Hesp and his co-workers,[4] (**2**) probably results from a novel cyclization of the pimaradiene cation (**3**) followed by Wagner–Meerwein rearrangement, but whether the final cyclization occurs on the α or the β face of the molecule, and the origin of the 8β-H, were

not resolved. We now report experimental data which clarify these questions.



SCHEME

Assignments of peaks in the natural abundance ^{13}C Fourier transform n.m.r. spectra of (**1**) and of the derived[4] 16-norketone (**4**) (Table), were based on chemical shifts[5] and on the multiplicities seen in offset-decoupled spectra, with the additional considerations noted in the Table. To

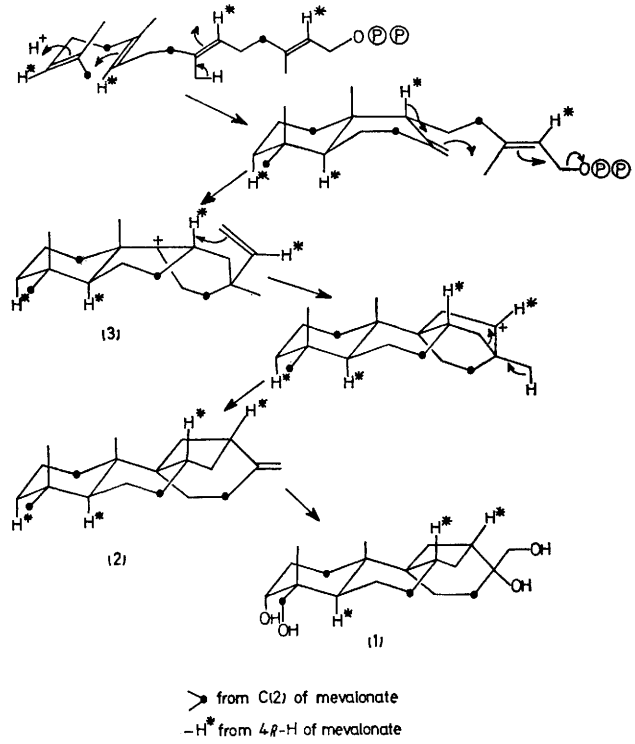
TABLE ^{13}C n.m.r. data.

C atom	δ^c (p.p.m. downfield from Me_4Si) and multiplicity: Aphidicolin (**1**) (in CD_3CO_2D)	16-Norketone (**4**) (in $CDCl_3$)
1 (or 7)[a]	27·4 (t)	26·4 (t)
3	76·9 (d)	76·9 (d)
4	41·2 (s)	40·6 (s)
5	41·7 (d)	41·3 (d)
7 (or 1)[a]	27·1 (t)	26·0 (t)
8	34·6 (d)	32·8 (d)
9	50·0 (s)	49·1 (s)
10	40·6 (s)	39·6 (s)
12	41·0 (d)	48·3 (d)
15[b]	28·3 (t)	34·5 (t)
16	77·3 (s)	207·2 (s)
17	68·1 (t)	—
18	17·8 (qt)	17·6 (qt)
19	71·5 (t)	71·5 (t)
20	15·5 (qt)	15·7 (qt)
2	33·5 (t)	34·0 (t)
6	32·1 (t)	31·7 (t)
11	27·6 (t)	26·7 (t)
13	25·3 (t)	22·6 (t)
14	23·9 (t)	21·6 (t)

[a] Both from mevalonate C(2) and so distinguished from unassigned CH_2 signals. [b] Signal disappears in spectrum of 15,15-$^2H_2$-(**4**).

clarify the biosynthesis of the bridged-ring system we used the principle that in an isoprenoid assembly labelled with paired ^{13}C atoms by incorporation of 1,2-$^{13}C_2$-acetate (pooled with an excess of unlabelled acetate), atoms derived *via* C(2) of mevalonate are unpaired and do not show ^{13}C–^{13}C coupling. Suitably-labelled material was obtained by feeding 0·33 mg ml^{-1} of a 3 : 1 mix of unlabelled and 1,2-$^{13}C_2$-acetate to 4-day shake cultures of *N-sphaerica*[3] and recovering 0·36 mg ml^{-1} of (**1**) 2 days later. To obtain a spectrum of the desired quality, this was converted into the more soluble (**4**). The ^{13}C n.m.r. spectrum of (**4**) showed five sharp singlets and 14 signals with strong satellites due to the ^{13}C–^{13}C pairs. Two of the five singlets were peaks which had not been assigned in the natural-abundance spectra but on general biosynthetic grounds can be identified as due to C(1) and C(7) (Table). A third was due to C(16), which is unpaired in the norketone (**4**) because C(17) has been removed; in the spectrum obtained directly for labelled (**1**) this peak was not a singlet. The other two singlets were unambiguously assigned to C(15) and C(19) which must therefore derive from C(2) of mevalonate.

The label in C(19) shows that in the formation of ring A, with its 10β-methyl, cyclization has occurred in the normal sense. That in C(15) establishes that the bridging attack of the vinyl residue on C(9) has occurred from the β-side, and makes it difficult to associate the origin of the 8β-H of (**1**) with this final cyclization.



Incorporation of 2-^{14}C-4*R*-^3H mevalonate (^3H:^{14}C in dibenzylethylenediamine salt 9·16 : 1, hereafter normalized as 4 : 4) gave (**1**) with 1·03 × 10^9 d.p.m. mol^{-1} ^3H and 1·50 × 10^8 d.p.m. mol^{-1} ^{14}C (normalized ^3H : ^{14}C = 3·0 : 4). Thus one of the four possible ^3H in (**1**) is missing. For the sense of ring A cyclization established by the ^{13}C data, one of these ^3H would be expected at 3α in (**2**) and will be absent in (**1**) if 3-oxygenation has occurred subsequently and with retention of configuration. This interpretation was confirmed by oxidation of (**1**) to the diketoacid (**5**),[4] with the expected loss of ^{14}C (found, 24·7%) from C(19) but no further loss of ^3H (normalized ^3H : ^{13}C = 3·0 : 3).

Conversion of (**1**) into (**4**) and of (**4**) into its 15,15-[$^2H_2$]-derivative (NaOD–D_2O in tetrahydrofuran at 15°; over 90% [$^2H_2$]- and no [$^2H_3$]-derivative by mass spectrometry) gave no loss of tritium (normalized ^3H : ^{14}C = 3·0 : 4), but conversion *via* (**4**) into the ketoacid (**6**)[4] gave a product with

normalized $^{3}H:^{14}C = 2{\cdot}0:4$. Thus one ^{3}H is located on C(12), rather than the alternative C(15), and this confirms the final cyclization deduced from the ^{13}C data.

Further degradation to locate the other two ^{3}H atoms unambiguously proved impracticable. However, the data show that the hydrogen originally attached to C(9) is retained in (**1**), and we thus conclude that this has furnished the 'extra' β-hydrogen at C(8). For such a migration to have occurred, a 4*R*-^{3}H-mevalonate-derived ^{3}H must have been situated on the β-face at C(9), which implies that, as in the biosynthesis of pleuromutilin,[6] ring B has originated from a boat folding of the geranylgeranyl precursor. Moreover it is not possible to write a mechanism in which hydride migration from C(9) to C(8) is concerted with the final cyclization since both processes are now seen to occur on the β-face of the molecule.

The biosynthesis, permitting both the hydride shift and a subsequent cyclization to occur on the same β-face, is shown in the Scheme. The side from which the final cyclization occurs is primarily determined by the stereochemistry at C(13) in the cation (**3**), which is that of a pimaradiene and not an isopimaradiene. Significantly, the mixtures of minor hydrocarbons co-occurring with (**1**) in *C. aphidicola*[4] and *N. sphaerica* contain, in addition to (**2**), at least two tricyclic dienes, and one of these has been positively identified as Δ8(9),15-pimaradiene (β-vinyl) by n.m.r.[7]

A variant of the scheme, with the alternative α-vinyl stereochemistry at C(13), also accounts for the structure of the plant product stemodin[8] with a carbon skeleton epimeric with that of (**1**) at C(9) and C(12) but still with the β-H at C(8) (*cf.* kaurene/phyllocladene).

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