The Biosynthesis of Asperlactone: Incorporation Studies with [2-^{13}C,2-^{2}H$_3$]Acetate

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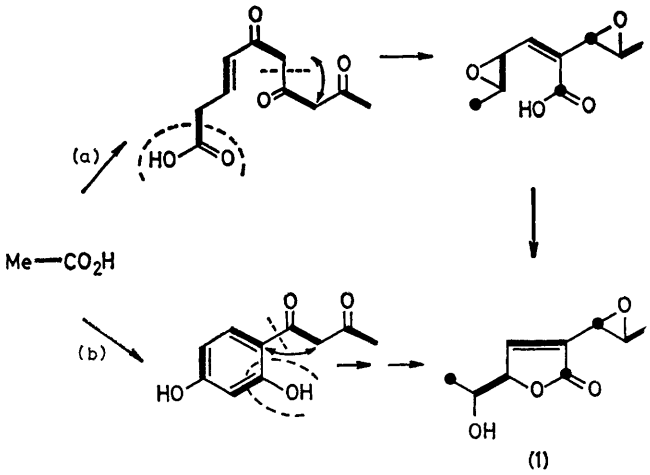
Summary The C-7 methyl group of asperlactone (**1**) retains up to two hydrogens from [2-^{13}C,2-^{2}H$_3$]acetate; the involvement of intermediates in which this carbon forms part of an aromatic ring is thus discounted.

RECENTLY, we showed that asperlactone (**1**) incorporates [1,2-^{13}C$_2$]acetate with the retention of three intact acetate units as shown in the Scheme.[1] Additionally, the detection of a two-bond ^{13}C–^{13}C coupling between C-2 and C-8 revealed that these two carbons are derived from a single

acetate unit.[2] AFavorskii-type mechanism can be proposed for this rearrangement which may take place either on a linear polyketide (path a) or on an aromatic intermediate (*e.g.* path b) as shown in the Scheme. We now describe experiments with [2-^{13}C,2-$^{2}H_3$]acetate[3] which help us to distinguish between these biosynthetic possibilities.



SCHEME

10 day old cultures of *Aspergillus melleus* IMI 49108 were refloated on to fresh sucrose-based medium (2 × 200 ml) and supplemented with [2-$^{2}H_3$,2-^{13}C]acetate (93 atom% ^{13}C, 99 atom% ^{2}H; 100 mg per flask per day over 5 days); on day 17, asperlactone (380 mg) was isolated. A parallel incorporation study with [1-^{14}C]acetate gave a 0·23% incorporation, corresponding to an estimated dilution factor per labelled site of 33.

In the 100 MHz proton noise decoupled ^{13}C n.m.r. spectrum of (**1**) in $CDCl_3$,† the signal for C-3 is enriched four-fold over natural abundance. The signals for C-5, C-7, C-8, and C-10, although enriched, are less intense than expected, consistent with the presence of some ^{2}H at these positions.

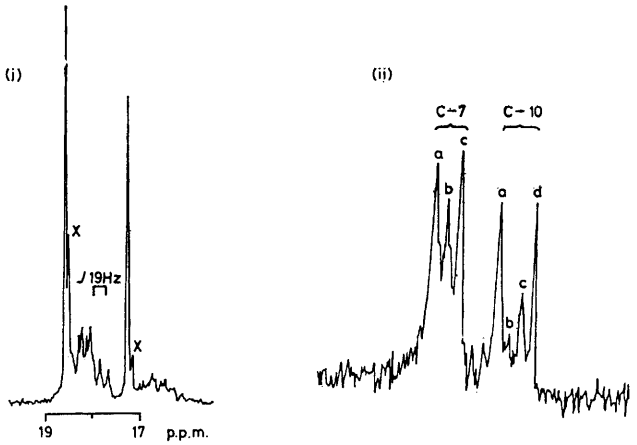


FIGURE. ^{13}C N.m.r. spectra of asperlactone (**1**): (i) 100 MHz ^{13}C {^{1}H}, (ii) 100 MHz ^{13}C {^{2}H, ^{1}H}; (a) $^{13}CH_3$, (b) $^{13}CH_2D$, (c) $^{13}CHD_2$, (d) $^{13}CD_3$.

The methyl region of the spectrum [Figure (i); Table] is complex; the strong singlets at δ 18·50 and 17·20 p.p.m. are clearly the normal $^{13}CH_3$ signals for C-7 and C-10 respectively. A triplet (*J* 19 Hz) centred at δ 18·22 p.p.m., 0·28 p.p.m. upfield of the main signal, and a quintet (*J* 19 Hz) centred at 17·96 p.p.m., 0·54 p.p.m. upfield, can be assigned to molecules labelled as $^{13}CH_2D$ and $^{13}CHD_2$ at C-7 respectively. A signal at δ 16·48 p.p.m. may be the centre resonance of a $^{13}CD_3$ septet for C-10 since it is 0·72 p.p.m. upfield of the normal signal for this carbon. Parts of the multiplets corresponding to $^{13}CHD_2$ and $^{13}CH_2D$ for C-10 are also visible.

These assignments were unambiguously confirmed by rerunning the spectrum‡ with simultaneous ^{1}H and ^{2}H decoupling [Figure (ii); Table]. Five singlets are clearly visible, assigned as follows: δ 18·59 ($^{13}CH_3$, C-7), 18·27 ($^{13}CH_2D$, C-7), 18·08 ($^{13}CHD_2$, C-7), 17·23 ($^{13}CH_3$, C-10), and 16·48 p.p.m. ($^{13}CD_3$, C-10). Weak signals between these last two are consistent with the presence of small amounts

TABLE. ^{13}C N.m.r. data for asperlactone (**1**) in $CDCl_3$.[a]

Carbon	Isotopic species	δ	Isotope shift (p.p.m.)	$J(^{13}C-^{1}H)$/Hz	$J(^{13}C-^{2}H)$/Hz
2	—	171·6(s)	—	—	—
3[b]	—	132·5(d)	—	—	—
4	—	147·8(s)	—	175	—
5	$^{13}C-^{1}H$	85·25(d)	—	151	—
	$^{13}C-^{2}H$	84·88	0·37	—	23
6	—	67·35(d)	—	143	—
7	$^{13}C-^{1}H_3$	18·50(q)	—	128	—
	$^{13}C-^{1}H_2{}^{2}H$	18·22	0·28	128	19
	$^{13}C-^{1}H^{2}H_2$	17·96	0·54	128	19
8[b]	—	52·00(d)	—	182	—
9	—	57·25(d)	—	176	—
10	$^{13}C-^{1}H_3$	17·20(q)	—	127	—
	$^{13}C-^{1}H_2{}^{2}H$	16·88	0·32	127	19
	$^{13}C-^{1}H^{2}H_2$	16·65	0·55	127	19
	$^{13}C-^{2}H_3$	16·48	0·72	—	19

[a] Recorded at 100 MHz on a Bruker WH-400 spectrometer. δ Values are in p.p.m. downfield of Me_4Si. [b] $J(^{13}C-^{13}C)$ 64 Hz.

† The free induction decay was recorded on a Bruker WH-400 spectrometer for a 0·87 M solution in $CDCl_3$ over an acquisition time of 0·8 s using a pulse angle of 40° to optimise the signal intensity of deuteriated carbons. The additional resonance in the Figure (i) arises from an isomer of (**1**) as discussed in ref. 1.

‡ Recorded for a 0·15 M solution in $CDCl_3$ containing 10% of C_6F_6. The slight differences in chemical shift values are due to the change in sample concentration and composition.

of $^{13}CH_2D$ and $^{13}CHD_2$ at C-10. There is no trace of a signal corresponding to $^{13}CD_3$ at C-7.

The proton-decoupled spectrum also reveals clearly a ^{13}C–^{2}H triplet for C-5 centred at δ 84·88 p.p.m., 0·37 p.p.m. upfield of the ^{13}C–^{1}H signal; with simultaneous decoupling, only two signals, singlets at δ 85·25 and 84·90 p.p.m., are visible. In contrast with C-5, C-8 shows no direct evidence for ^{2}H retention apart from slightly reduced signal intensity. Both C-8 and C-3 show flanking satellites [J(^{13}C–^{13}C) 64 Hz] resulting from the presence of ^{13}C at the adjacent carbon.

The retention of two acetate-derived hydrogens rules out the intervention of aromatic intermediates in which C-7 forms part of an aromatic ring. Taken with the results of the [1,2-$^{13}C_2$]acetate study, this leaves path (a), involving no aromatic intermediate, as the most plausible pathway for asperlactone biosynthesis.

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[2] For related work on aspyrone, a co-metabolite of asperlactone, see T. J. Simpson and J. S. E. Holker, *Tetrahedron Lett.*, 1975, 4693; M. Tanabe, M. Uramoto, T. Hamasaki, and L. Carey, *Heterocycles*, 1976, 355.
[3] M. J. Garson and J. Staunton, *Chem. Soc. Rev.*, 1979, 539.