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THE ^{13}C NMR SPECTRUM OF A PYRONE METABOLITE OF *ASPERGILLUS MELLEUS*. BIOSYNTHETIC INCORPORATION OF SINGLY AND DOUBLY LABELLED [^{13}C]-ACETATE.

Thomas J. Simpson[1]

Department of Organic Chemistry, The Robert Robinson Laboratories,

P.O. Box 147, Liverpool L693BX

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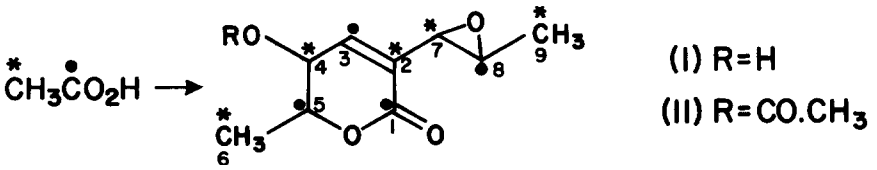
Fermentations of *Aspergillus melleus* provide a variety of metabolites; the mycelium is a rich source of naphthaquinones[2] whereas the principal metabolite in the liquors is the pyrone (1)[3]. Incorporation of [^{14}C]-acetate into pyrone (1) is reported[4] to give the labelling pattern shown (Scheme 1). This distribution of label, particularly the linkage of two carbons derived from the methyl of acetate, is difficult to rationalise in terms of the normal pathways of polyketide biosynthesis. Studies based on ^{13}C-labelling especially the use of doubly labelled [^{13}C]-acetate have facilitated the elucidation of unusual biosynthetic pathways[5,6]; thus samples of pyrone (I) enriched biosynthetically with [1-^{13}C]-, [2-^{13}C]- and [1,2-^{13}C]-acetate have been prepared and their ^{13}C spectra determined.

A full assignment of the ^{13}C n.m.r. spectra of pyrone (I) and its acetate (II) (table) has been made as follows:- C_1, C_2, C_3 and the C_4-acetate group were readily assigned from known chemical shift data[7] and their multiplicities in the off-resonance-decoupled spectra. C_4, C_5, C_7 and C_8 all have chemical shifts characteristic of oxygen-bearing aliphatic carbons and give doublets in the off-resonance spectra. They were distinguished in the acetate (II) by plotting the peak frequencies in the off-resonance spectra against the ^{1}H irradiating frequency as it is stepped through the ^{1}H spectral region[8] (figure 1). The ^{1}H resonance

Scheme 1. Incorporation of [^{14}C]-acetate into *A. melleus* pyrone.



frequency corresponding to the carbon shift is determined when the residual coupling goes to zero. The method is only applicable in this case as the ^{1}H frequencies in acetate (II) are well separated and have been unambiguously assigned[3].

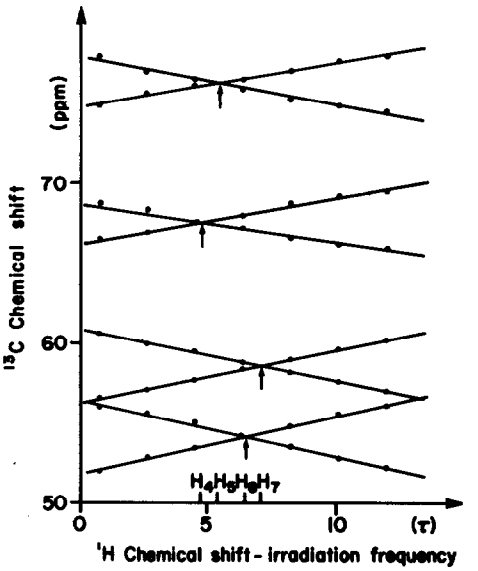
The C_6 and C_9 methyl signals could not be readily distinguished in the natural abundance spectra. However, in the spectrum of (I) enriched with [1,2-^{13}C]-acetate, C_9 shows a strong ^{13}C-^{13}C coupling with C_8 (see below).

Table:

^{13}C Chemical Shifts of Pyrones (I) and (II)

carbon	(I)	(II)
1	163.0	161.7
2	129.0	131.2
3	141.2	135.5
4	67.6	67.8
5	79.3	76.3
6	18.0	18.1
7	54.6	54.3
8	59.1	58.7
9	17.6	17.5
$\underline{CH_3}CO$	-	20.6
$CH_3\underline{CO}$	-	169.4

Figure 1



In the ^{13}C n.m.r. spectrum of (I) labelled from [1-^{13}C]-acetate (fig 2a) C_1, C_3, C_5 and C_8 exhibit strong enhancement relative to natural abundance, whereas the [2-^{13}C]-acetate enriched spectrum (fig 2b) shows enhancement of C_2, C_4, C_6, C_7, and C_9 and a ^{13}C-^{13}C coupling of 61Hz between C_2 and C_7 indicative of a head-to-head linkage of acetate groups. This confirms the previously reported labelling pattern[4]. In addition, the [1,2-^{13}C]-acetate enriched spectrum (fig 2c) shows intense couplings between C_2-C_3, C_4-C_5, and C_8-C_9 of 68,41 and 44Hz, respectively indicating their origin from intact acetate units. Due to the high enrichment, couplings of 61, 42, 40, and 32Hz between C_2-C_7, C_3-C_4, C_5-C_6 and C_7-C_8 respectively were seen as weak intesntiy lines indicating their origin from adjacent acetate units. This is believed to be the first assignment of the low intensity couplings in a [1,2-^{13}C]-acetate enriched spectrum.

The absence of an intense coupling clearly indicates that the C_6 methyl cannot be part

of a "starter" unit in the biosynthesis of pyrone (1). A pathway which would account for the observed incorporation of acetate is given in Scheme 2. In this context it is notable that mellein which represents an alternate folding of the pentaketide chain is a co-metabolite of (I) and on repeated culture of *A. melleus*, yields of pyrone (I) decreased with concomitant increase in mellein production[3]. Penicillic acid, known to be formed by ring cleavage of orsellinic acid in *Penicillium patulum*[9] is also a co-metabolite. A Favorskii-type rearrangement would account for the head-to-head linkage of acetate units. A head-to-head linkage in polyketide metabolites has previously only been observed in the aflatoxins and related compounds[10,11]. The mechanism proposed to account for this[10] is without precedent amongst known chemical rearrangements and must clearly be in doubt with the recent incorporation[12] of averufin into aflatoxin B_1.

Further studies to provide evidence for the above postulates are in progress.

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Scheme 2. Postulated biosynthesis of pyrone (I) via a pentaketide precursor.

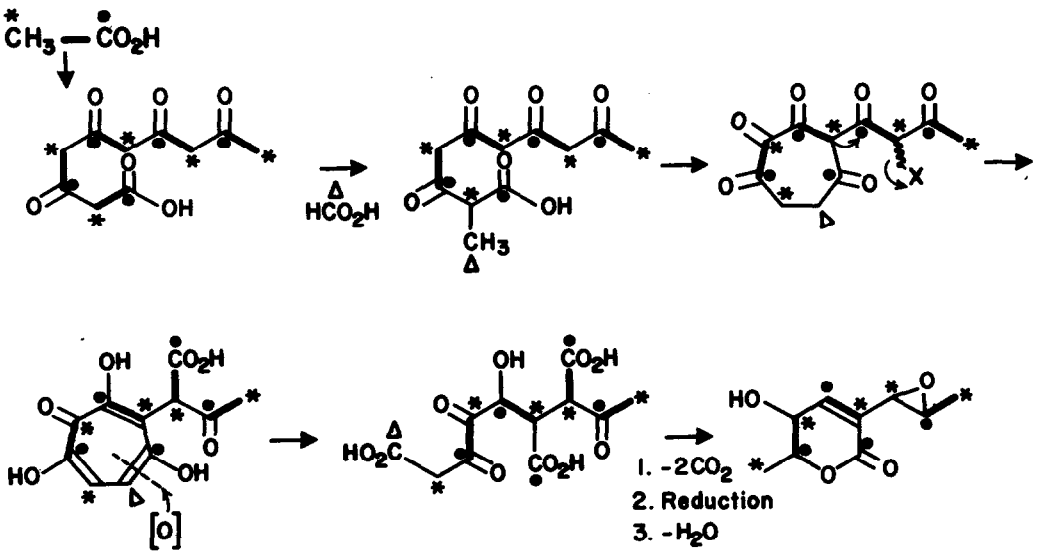

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Figure 2. ^{1}H noise de-coupled ^{13}C n.m.r. spectra of pyrone (I) from (a) $CH_3{}^{13}COONa$, (b) $^{13}CH_3COONa$, and (c) $^{13}CH_3{}^{13}COONa$.