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THE BIOSYNTHESIS OF A PYRONE METABOLITE OF *ASPERGILLUS MELLEUS* AN APPLICATION OF LONG-RANGE ^{13}C-^{13}C COUPLING CONSTANTS

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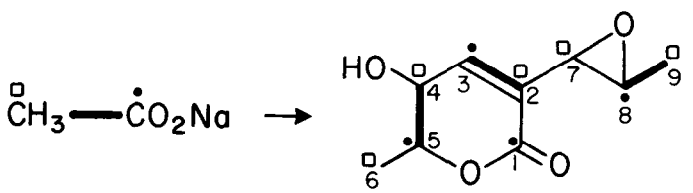
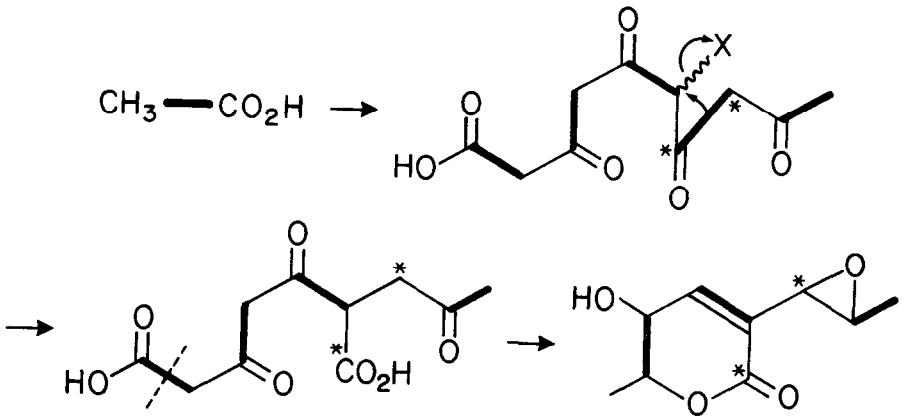
The pyrone (1), a weak broad-spectrum antibiotic, has been isolated from *Aspergillus* species.[1,2] ^{14}C-Labelling studies[3] suggest a polyketide origin, and incorporation of [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C]acetate into the pyrone by static cultures of *A. melleus* indicated its formation from three intact acetate units and three carbons derived from cleaved acetate units. A biosynthetic pathway involving cyclisation and rearrangement of a pentaketide precursor followed by ring cleavage, was suggested to account for this most unusual labelling pattern.[4]



(1)

An alternative pathway, involving rearrangement of a pentaketide precursor followed by loss of the terminal carboxyl as shown in the scheme, would also account for the observed labelling pattern. In the course of this rearrangement, an originally intact acetate unit is cleaved and so the 1,2 coupling should be lost, as is observed in the ^{13}C n.m.r. spectrum of the [1,2-^{13}C]acetate enriched pyrone (Table). However, the respective carbons are now in a 1,3 relationship and so, if this is the correct pathway, there should be a two-bond ^{13}C-^{13}C



SCHEME. Proposed biosynthesis of pyrone (1) *via* a pentaketide precursor.

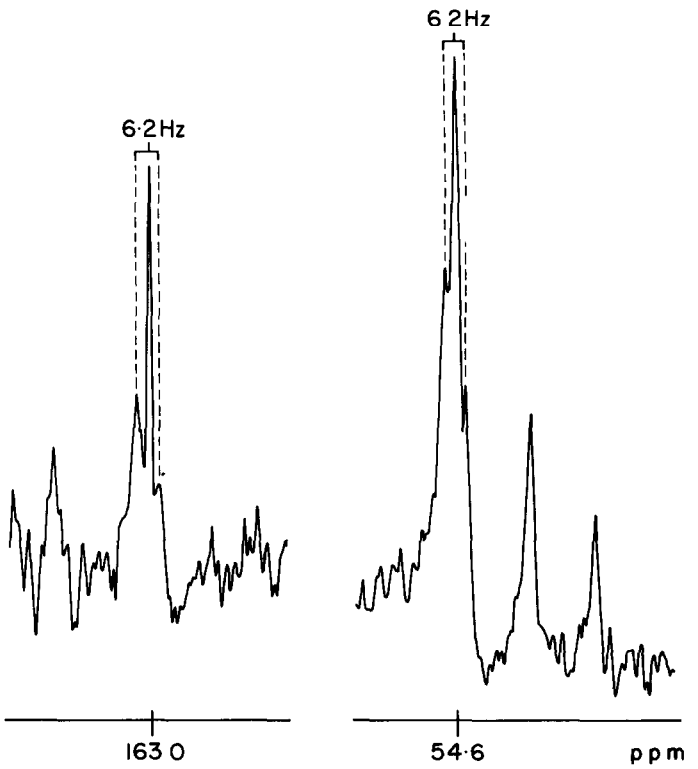


FIGURE. ^{13}C N.m.r. spectrum of [1,2-^{13}C]acetate enriched pyrone (1).

Determined on Varian XL-100 operating at 25.2 MHz., 500 Hz total sweep widths.

coupling between C-1 and C-7. Two-bond ^{13}C-^{13}C couplings are small, typically 0-10 Hz,[5] and can be difficult to resolve,[6] but should provide a method for differentiating between the two possible biosynthetic pathways.

A closer examination of the [1,2-^{13}C]acetate enriched spectrum revealed a significant broadening of the C-1 and C-7 resonances, relative both to the other carbon resonances in the spectrum and to the C-1 and C-7 resonances in unenriched spectra. Thus the ^{13}C n.m.r. spectrum of the [1,2-^{13}C]acetate enriched pyrone was redetermined using 500 Hz total sweep widths. A ^{13}C-^{13}C coupling of 6.2 Hz (see Figure) between C-1 and C-7 is apparent, so providing conclusive evidence for the biosynthetic pathway shown in the scheme. There is no established biosynthetic precedent for this pathway, but a similar rearrangement can be postulated to account for the formation of the fused difuran ring system, found in the aflatoxins and related metabolites, whose origin has been a subject of much speculation.[7]

This is believed to be the first example of detection of a long-range ^{13}C-^{13}C coupling arising from biosynthetic rearrangement of a doubly ^{13}C-labelled precursor. Two-bond ^{13}C-^{13}C couplings have been detected due to very high incorporations of [1-^{13}C]-acetate into aflatoxin B_1,[8] and intramolecular rearrangement of [2,11-^{13}C]porphobilinogen during the course of

TABLE. ^{13}C N.m.r. chemical shifts (p.p.m.) and coupling constants (Hz) in [1,2-^{13}C]acetate enriched pyrone (1)

Carbon	δ	$^{1}J_{C-C}$	$^{2}J_{C-C}$
1	163.0	-	6.2
2	129.0	68	-
3	141.2	68	-
4	67.6	41	-
5	79.3	41	-
6	18.0	-	-
7	54.6	-	6.2
8	59.1	44	-
9	17.6	44	-

protoporphyrin biosynthesis gives rise to a one-bond ^{13}C-^{13}C coupling.[9] The method has potential application to the terpenoid field where the path of methyl and other bond migrations may be followed, as well as to the study of molecular rearrangements in general.

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