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THE BIOSYNTHESIS OF 2,5,6-TRIHYDROXY-9-PHENYLPHENALENONE BY LACHNANTHES TINCTORIA. INCORPORATION OF 1-^{13}C-PHENYLALANINE.[1]

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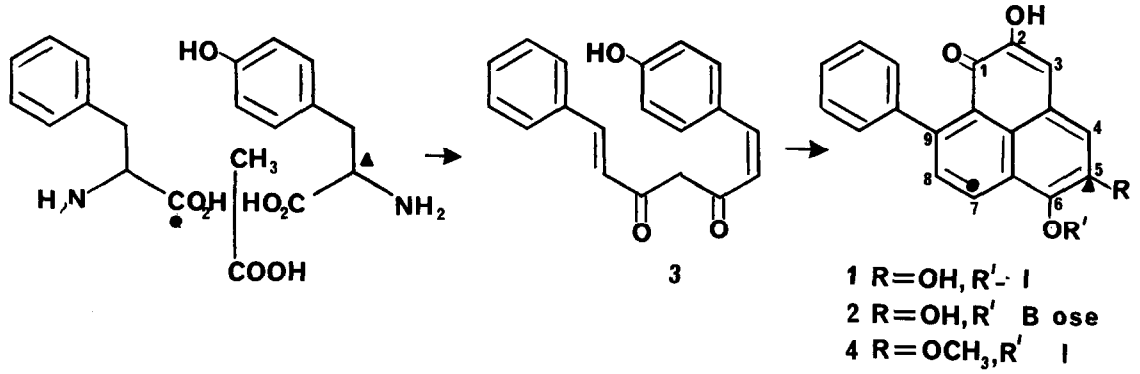
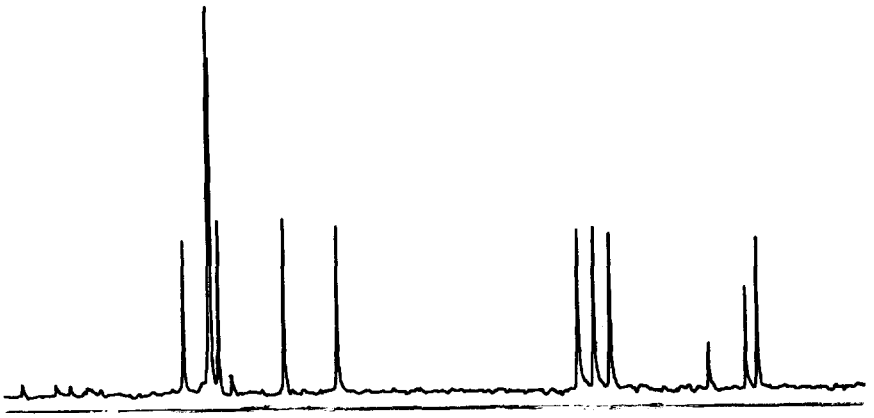
2,5,6-Trihydroxy-9-phenylphenalenone (1) is the aglycone of lachnanthoside (2), the major pigment isolated from the root-system of Lachnanthes tinctoria (Haemodoraceae).[2] In 1961, Thomas[3] suggested that the biosynthesis of the 9-phenylphenalenones might proceed through a sequence involving the unique combination of one mole each of phenylalanine and tyrosine, and one mole of acetic acid (Scheme); oxidative cyclization of the intermediate biarylheptanoid (3)[4] would lead directly to the natural products. Biosynthetic studies using ^{14}C-labelled precursors have established the ready incorporation of phenylalanine, tyrosine, and acetate into 1 by L. tinctoria,[5] and into haemocorin aglycone (4) by Haemodorum corymbosum;[6] significantly, Thomas[6] observed the exclusive incorporation of label from 2-^{14}C-tyrosine into C-5 of 4, an observation which lent considerable support to the proposed biosynthetic scheme.



The preliminary biosynthetic studies undertaken with L. tinctoria[5] showed a dramatic (3.8%) incorporation of 1-^{14}C-phenylalanine into 1. This, coupled with the lack of suitable degradative techniques which would allow access to the carbons thought to arise from phenylalanine (viz. the 9-phenyl substituent, and carbons 7, 8 and 9), led us to an investigation based upon the incorporation of a ^{13}C-labelled substrate.

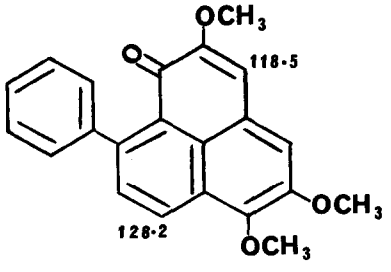
1-^{13}C-Phenylalanine (89 atom% excess ^{13}C determined by MS[7]) was prepared from 1-^{13}C-glycine by a combination of the methods of Kirby and Michael,[8] and Strange, et al.[9] in 36% yield. 150 mg of the ^{13}C-enriched amino acid together with 0.06 mg of 1-^{14}C-phenylalanine (4.6×10^7 dpm) was fed to 36 prewashed, growing, Lachnanthes plants through the roots. After 3 days, analysis of the feeding solution showed that 34% (51 mg) of the phenylalanine had been taken up by the plants. Lachnanthoside aglycone was isolated, and converted into the two isomeric trimethylethers (5 and 6) as previously described[5] (29.5 mg, 1.3×10^7 dpm/mmole, 26% specific incorporation[10]). This result indicated that 6 mg of the ^{13}C-labelled substrate had been incorporated into the isolated 1. Mass spectrometric analysis of the ^{13}C incorporation into 6 indicated the presence of 10.9% of the mono-labelled species. There is no explanation for the apparent discrepancy between the incorporation data obtained by liquid scintillation counting and MS analysis.

Examiaation of the proton noise decoupled CMR spectra of 6 at natural abundance and with ^{13}C-enrichment, showed a significant peak enhancement at 128.8 ppm in the enriched sample. We have been unable to assign unequivocally all the resonances in the CMR spectrum of 6, however, that at 128.8 ppm, which occurs as a doublet (J = 160 Hz) when undecoupled, was readily correlated with C-7 by single proton decoupling experiments. The proton at C-7 is the most deshielded resonance in the PMR spectrum and resonates at 8.57 ppm.[2,11] The peak heights of the CMR signals were normalized to that of C-4 (118.5 ppm), and a peak height ratio was calculated for each resonance (Table). An enhancement of 320% was observed at 128.8 ppm in the ^{13}C-enriched 6. Complementary deductions could be made from the spectra of the isomeric ether 5 (see Figure); the enriched peak (relative peak height increase 500%) was at



Natural-Abundance CMR Spectrum of Trimethyl Ether (5); 15 mg in 2.5 ml $CDCl_3$; 44816 Transients, 20° Pulse.

CMR Spectrum of ^{13}C-Enriched Trimethyl Ether (5); 14.8 mg in 2.5 ml $CDCl_3$; 47919 Transients; 30° Pulse.



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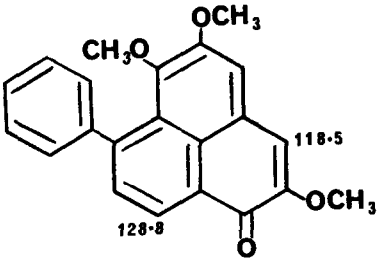

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128.2 ppm which is again consistant with the specific incorporation of ^{13}C label into carbon 7. We feel that these observations provide significant support for Thomas' hypothesis concerning the biosynthesis of the plant phenalenones.

Table. CMR of Biosynthetic 6.

Chemical Shift	Relative Peak Height Natural abundance/ ^{13}C-Enriched
130.9	99/100
128.8	153/642
128.7	
127.1	218/202
126.8	135/128
124.9	
118.3	100/100
113.0	
60.8	47/ 39
56.9	
55.6	88/ 92

Determined on a JEOL FX-60 nmr spectrometer at 15 MHz; 8K data points over 1000 Hz were used with 1.2 sec pulse repetition rate and 30 pulses. Both spectra were obtained on ca. 0.02 M solutions.

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10. Specific incorporation is defined as the specific radioactivity found in the metabolite divided by the specific radioactivity of the precursor that was fed.

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