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THE CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTRUM OF SIDERIN

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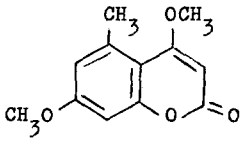
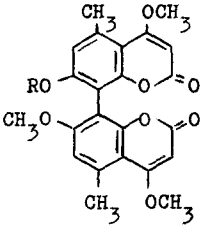
Recently the fungal metabolite 4,7-dimethoxy-5-methyl coumarin (I) has been isolated[1] from the petroleum ether extract of Aspergillus variecolor (IMI 53749). Its structure has been established by chemical and spectroscopic methods and by the carbon-13 nmr investigation reported here. Its synthesis has also been achieved[1,3]. This compound, which has been given the trivial name, siderin[2,3], has been reported to occur in the plants Sideritis canariensis[2] and Sideritis romana[3], and Buchi et al.[4] have also reported the presence of its dimers, kotanin and desmethylkotanin (II), from Aspergillus clavatus.



(I)

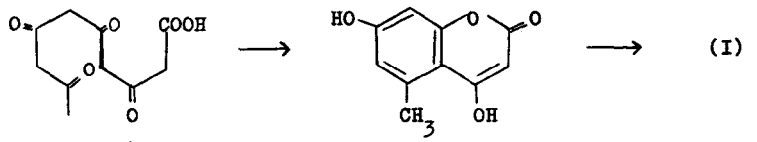
Siderin



(II) a) R = CH_3 Kotanin

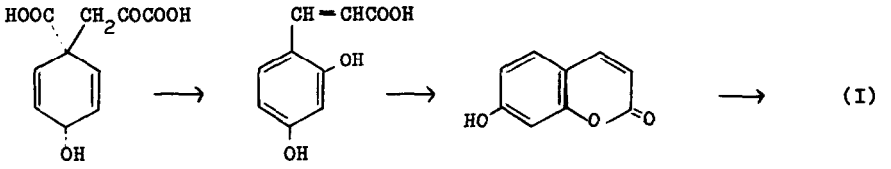
b) R = H Desmethylkotanin

Attention has now been focussed on the biosynthetic origin of siderin. Biosynthesis may well occur via a polyketide chain containing five units (III) with subsequent transformation involving no change in oxidation state and methylation terminating the synthesis. Turner[5] has however suggested that uncyclized residues from the methyl end of polyketide chains are never shorter than residues from the carboxyl end and therefore the possibility of such a cyclization as envisaged above might be ruled out.



(III)

A further possible biosynthetic origin is via the shikimic acid pathway. Although such a route plays a smaller role in the secondary metabolism of fungi than the polyketide pathway, siderin can theoretically be derived from prephenate with C-methylation and 4-hydroxylation occuring at a later stage thus:

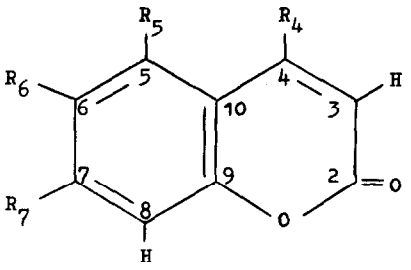


It is of great interest to establish which pathway is operative in the biosynthesis. Indeed, it may be that the polyketide route is operative in the fungal product whereas the same compound arises in the higher plants on the shikimic acid pathway.

The prerequisite of the study of the biosynthesis of siderin by carbon-13 labelling experiments and nmr is that a complete unambiguous assignment of the natural abundance carbon-13 nmr spectrum must be made. To this end a series of coumarin derivatives have been investigated.

The proton noise and single frequency off-resonance decoupled carbon-13 nmr spectra were obtained on a Varian Associates XL-100-15FT spectrometer at 25.197 MHz. Solutions of compounds (IV) - (VIII) were 0.7 to 1.0 M in acid-free deuteriochloroform with 2% tetramethylsilane (TMS) added as internal reference. In the case of siderin (I) low solubility allowed only about a 0.1 M solution to be used.

The carbon-13 resonances in the spectra of compounds IV to VIII were assigned to specific carbons by the following procedures: a) the number of protons attached to each carbon was determined by proton off-resonance decoupling; b) the



I R_6=H, R_4=R_7=OMe, R_5=Me

IV R_4=R_5=R_6=R_7=H

V R_4=R_5=R_6=H, R_7=OMe

VI R_4=R_5=R_7=H, R_6=Me

VII R_5=R_6=H, R_4=R_7=OMe

VIII R_4=R_6=H, R_5=Me, R_7=OMe

TABLE

Carbon-13 Chemical Shifts[+] of some Coumarin Derivatives in $CDCl_3$ Solution

Carbon	I	IV	V	VI	VII	VIII
2	162.7*	159.9	160.5	160.2	162.6	160.5
3	87.4	116.3*	112.1*	116.0	87.3	111.8
4	169.3	142.8	142.9	142.9	166.2	139.8
5	138.2	127.4	128.3	127.3	123.6	136.7
6	115.4	123.9	112.6*	133.6	111.6	113.4
7	161.6*	131.3	162.2	132.2	162.6	161.7
8	98.6	116.4*	100.5	116.0	100.1	98.4
9	156.3	153.4	155.4	151.6	154.5	155.9
10	107.6	118.4	112.2	118.1	108.5	111.1
4-OMe	55.8	-	-	-	56.1	-
7-OMe	55.4	-	55.6	-	55.6	55.4
5-Me	23.4	-	-	-	-	18.4
6-Me	-	-	-	20.6	-	-

[+] In ppm downfield from TMS (accurate to 0.1 ppm) : * Assignments may be reversed.

methoxy and methyl resonances were assigned by their characteristic chemical shifts in comparison with literature values [6,7]; and c) the use of substituent chemical shift (SCS) additivity principles for methyl and methoxy substituents on a benzene ring and on olefinic systems to give a total assignment of the coumarin ring chemical shifts for all the compounds.

Having derived a self-consistent set of shifts for IV - VIII it was possible to obtain SCS effects for 4- and 7- methoxy and 5-methyl substitution and hence predict the carbon-13 chemical shifts for siderin (I). The observed shifts were in

good agreement with those predicted except in the case of the C-4 and C-5 shifts, which were both about 6 ppm to higher field than calculated, and the 5-methyl shift which was about 5 ppm to low field of that in compound VIII. These discrepancies are however those which might reasonably be expected in view of the anticipated 4-methoxy/5-methyl steric interactions and resulting geometry distortions of the coumarin ring; such large effects have been observed in 1,8-dimethylnaphthalene [8,9] for example. Such a self-consistent fit of the carbon-13 chemical shifts for the compounds studied in itself clearly confirms the substitution pattern in siderin.

The chemical shift assignments in the molecules studied are given in Table.

Recently an investigation using carbon-14 tracer techniques has suggested that the fungal metabolite, siderin, is derived on a β-ketide pathway[1]. Research involving carbon-13 enriched sodium acetate feeding studies to establish the biosynthetic pathway to fungal metabolites of this type is currently being carried out.

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