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THE CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTRUM OF JUNIPAL

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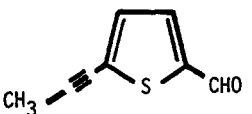
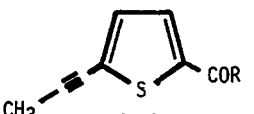
Junipal (I), a neutral metabolite isolated from Daedalea juniperina Murr., has been known[1] for twenty years and, until recently, represented the only known fungal polyacetylenic thiophen. Re-investigation of the fungus has however revealed[2] the presence of two further thiophens (II, R = $CH(OH)CH_3$) and (II, R = $COCH_3$). The rationale for the structure of these novel metabolites has come from spectroscopic methods and chemical synthesis[2].



(I)



(II)

Biosynthetic studies using ^{14}C-labelled substrates have shown[3] that junipal, in common with other C_8-polyacetylenes, may arise via the crepenynate pathway with the terminal eight carbons of crepenynate providing the carbon skeleton (Scheme). Bohlmann[4] has concluded from biosynthetic studies with higher plants that thio-enol ethers can act as intermediaries in the biogenesis of thiophen metabolites. This is an attractive hypothesis for the origin of junipal, although the detailed biomechanisms involved in the ring cyclisation and oxidative loss of the two proximal carbon atoms from a dehydromatricaria ester precurser remain to be clarified. In the tribus Anthemideae, which is well known[4] for its ability to elaborate a range of polyacetylenic thiophen metabolites derived from dehydromatricaria ester, the common presence of β-2-(5-propynyl)-thienylacrylic acid would suggest that cyclisation in higher plants, and possibly also in fungi, occurs at the C_{10} stage prior to the function group modifications.

Scheme

Oleate

↓

Crepenynate

↓

$*CH_3.C{\equiv}C.C{\equiv}C.C{\equiv}C.CH_2.CH{=}^{c}CH(CH_2)_7.CO.OR$

↓

$*CH_3.C{\equiv}C.C{\equiv}C.C{\equiv}C.CH{=}^{t}CH.CO.OR$

↓

(I, $*CH_3$.)

Recently, biosynthetic studies have taken on a new dimension in the shape of PFT-^{13}C spectroscopy since the necessity of degradation to locate a specific label is removed. We have been interested in utilising carbon-13 n.m.r. as an aid to structure determination and an adjunct to traditional carbon-14 biogenetic studies in the polyacetylene field. The prerequisite for the study of the biosynthesis of junipal by carbon-13 labelling experiments and n.m.r. is that a complete assignment of the natural abundance carbon-13 n.m.r. spectrum must be made. To this end, a series of thiophen derivatives and model compounds have been investigated. The chemical shift data are found in the Table.

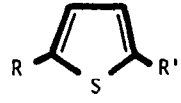
The assignment of the spectral peaks is based on several well-established methods which are now common in ^{13}C spectroscopy[5,6]. These included chemical shift theory, multiplicity patterns obtained from off-resonance experiments, intensity data and, for the acetylenic carbons, the observation[7,8] that the magnitude of the proton-carbon spin-spin coupling for a β-disposition is greater (approx. three times) than the γ-coupling along a substituted acetylenic chain.

The structural assignment of the junipal ring carbons was especially facilitated by comparison with thienyl- and furyl-acrylates. The olefinic carbon shieldings of the thienylacrylates mimic trends observed[9] for substituted α,β-unsaturated methyl esters with the π-polarisation resulting in the strong deshielding of the β-carbon. The most pronounced effect of the acrylate substituent on the ring carbons is the deshielding of C_2 by ca. 15 ppm relative to thiophen. A similar downfield shift of C_2 is also found for 2-formyl- and 2-acetyl-thiophen with the chemical shift data closely following patterns recently reported[10,11,12] for 2-substituted five membered aromatic heterocycles allowing for solute-solvent interactions. In accord with resonance effects and charge delocalisation, C_4 is the least affected in the 2-monosubstituted compounds with the substituent chemical shift effects roughly parallelling shieldings observed[13] in benzene derivatives. It is interesting also to note that the relative deshieldings of the α-carbons ($C_{2,5}$) to the β-carbons ($C_{3,4}$) in the 2,5-disubstituted compounds is similar to that found in the corresponding furan, e.g. wyerone derivatives[8], although the magnitude of the difference is smaller.

In junipal, the propynyl substituent induces a diamagnetic shift[14,15] at the attached carbon when compared to the corresponding alkyl or acrylyl substituent. Relative to ethynyl benzenes however, this shielding effect is considerably reduced (5 ppm compared to 15 ppm) which presumably is due to the electronic interactions between the sulphur atom and the 2-substituent. The polarising effect of the sulphur atom is also reflected in the chemical shifts of the propynyl carbons themselves. Compared to the corresponding methyl phenylacetylene[16] the thienyl ring results in a deshielding of the α-carbon by 10 ppm but a shielding of the β-carbon by 5 ppm. This chemical shift difference between the α- and β-substituent carbons is indicative[15] of considerable polarisation in the acetylenic bond.

The ^{13}C n.m.r. spectra of these substituted thiophens thus show that each carbon of junipal can be readily assigned and that the thiophen ring ^{13}C chemical shifts follow the same additivity patterns as other heterocyclic aromatics. Research to elaborate further additivity parameters in 2,5-disubstituted thiophens and the application of carbon-13 enriched substrates to the biosynthesis of fungal metabolites of the junipal type is currently under investigation.

Carbon-13 Chemical Shifts* of Junipal and Related Thiophens



Compound		Ring Carbons				R-Carbons				R'-Carbons	
R	R'	2	3	4	5	1	2	3	4	1	2
$CH_3.C{\equiv}C$	CHO	134.0	131.7	136.1	142.8	95.9	72.8	4.9		182.4	
$CH_3O_2C.CH{=}CH$	H	139.5	130.9	128.0	128.4	137.2	116.4	167.0	51.5		
$CH_3O_2C.CH{=}CH$	CHO	144.5	130.7	135.8	147.3	136.4	120.6	166.1	51.7	182.6	
$CH_3O_2C.CH{=}CH$	$CH(OCH_3)_2$	144.9	130.6	126.2	139.5	137.2	116.5	166.9	51.4	99.6	52.4
CHO	H	143.7	136.6	128.3	135.0	183.0					
$COCH_3$	H	144.5	132.6	128.2	133.8	190.7	26.8				

* The proton noise and single frequency off-resonance decoupled carbon-13 n.m.r. spectra were obtained on a Bruker HFX multinuclear spectrometer operating at 22.63 MHz. The compounds were measured as 20 - 25% (w/w) solutions in deuterochloroform containing ca. 5% Me_4Si. The data recorded are in p.p.m. downfield from the internal tetramethylsilane. The carbon atoms in the substituted thiophens are numbered from point of attachment.

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