The Carbon-13 Nuclear Magnetic Resonance Spectra of the Antibiotic Polyacetylenic Nitrile, Diatretyne 2 (7-Cyanohept-*trans*-2-ene-4,6-diynoic Acid), and Related Compounds

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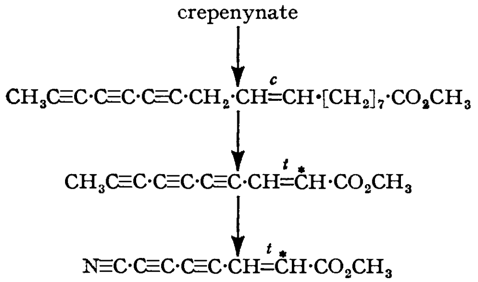
The natural abundance carbon-13 chemical shifts of the nitrile ester (9; R = CH_3) (diatretyne 2 methyl ester) and a series of related acetylenic compounds have been measured and correlated with structure. The effect of the various substituents and the carbon–carbon triple bond on the chemical shift of the immediate carbon nuclei is discussed.

ANTIBIOTIC activity has been reported for a number of naturally occurring mono- and poly-acetylenic compounds with the physiological activity depending on the relationship and array of the functional groups present in the molecule. Several members of the Tricholomataceae sub-family, a division of the family Agaricaceae, elaborate[1] a range of polyacetylenes including the fungicidal and bactericidal nitrile acid, 7-cyanohept-*trans*-2-ene-4,6-diynoic acid (9; R = H), which Anchel christened diatretyne 2.[2] The structure of this novel metabolite has been fully established by chemical and spectroscopic methods and confirmed by synthesis.[3]

Nitriles, particularly polyacetylenic nitriles, are very restricted in nature. The ability of surface cultures of *Lepista diemii* Singer.[4] or *L. glaucocana* (Bres) Singer.[5] to produce high concentrations of the nitrile acid (9; R = H) and the co-occurring diacid (10; R = H) and hydroxy-acid (13; R = H) provides an opportunity to study their biogenesis and the origin in these fungi of the cyano-group. Using ^{14}C-labelled substrates, incorporation studies have revealed[6] that the polyacetylenic skeleton of the nitrile acid (9; R = H) may arise *via* the crepenynate–dehydromatricaria ester pathway (Scheme). Despite extensive substrate feeding experiments, the origin of the nitrile carbon atom and the mechanism of loss of at least two distal carbons from a dehydromatricaria percursor remain, however, obscure.



$$\begin{tikzcd}[row sep=small] \text{crepenynate} \arrow[d] \\ CH_3C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH_2{\cdot}CH\overset{c}{=}CH{\cdot}[CH_2]_7{\cdot}CO_2CH_3 \arrow[d] \\ CH_3C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}\overset{*}{C}H{\cdot}CO_2CH_3 \arrow[d] \\ N{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}\overset{*}{C}H{\cdot}CO_2CH_3 \end{tikzcd}$$

SCHEME

The rapid advances in ^{13}C spectroscopy and particularly the application of Fourier transform techniques have added a new dimension to biogenetic studies since the necessity of extensive chemical degradation of labelled compounds no longer arises. In common with other polyacetylenes, the degradation and localisation of the acetylenic carbons of diatretyne 2 presents special

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difficulties with only the proximal first two carbons readily accessible.[6] The prerequisite for a study of the biosynthesis of the nitrile acid (9; R = H) and associated metabolites by carbon-13 labelling experiments is that a complete assignment of the natural abundance ^{13}C spectra of these compounds must be made. In view of the limited data available on the ^{13}C chemical shifts of polyacetylenes, and the potential this spectroscopic method offers for polyacetylene biosynthesis, a series of compounds related to the nitrile ester (9; R = CH_3) has been investigated.

RESULTS AND DISCUSSION

The chemical shifts of the *sp*-hybridised carbons of simple alkynes extend [7,8] over the range *ca.* 26—65 p.p.m. They occur in a very diagnostic region of the ^{13}C spectral range with very limited overlap with most other functionalised carbon types. However, they are very sensitive to substituent effects particularly those involving $(p-d)\pi$ or $(p-p)\pi$ electronic interactions. In simple conjugated alkenynes, it has been shown [9] that the double bond causes a strong deshielding of the β-acetylenic carbon and a weak shielding of the α-acetylenic carbon compared with the corresponding alkyl substituent. Bohlmann and Zeisberg have recently reported [10] a study of symmetrical poly-ynes and a series of Compositae spiroacetalenol ethers believed to be derived biogenetically from trideca-*trans*-4-ene-7,9,11-triyn-1-ol. In these compounds, which possess the substituted enyne linkage, the deshielding of the β-acetylenic carbon by the olefinic bond was again observed.

Carbon-13 chemical shifts * of diatretyne 2 and related compounds

Compound	1	2	3	4	5	6	7	8	9	10	11	12
(1)	83.1	81.8	176.8									
(2)	73.8	82.0	50.4									
(3)	73.5	79.2	91.0	60.9	15.1							
(4)	74.7	57.1	104.7									
(5)	3.9	74.4	65.4	68.8	64.7							
(6)	4.0	72.2	64.8	64.8	72.2	4.0						
(7)	78.2	81.8	108.9	143.7	62.1							
(8)	86.1	80.2	132.1	124.3	165.9	51.9						
(9; R = CH_3)	104.5	56.6	65.8	78.4	78.0	137.8	120.8	165.2	52.0			
(9; R = CH_3) [a]	105.2	56.2	65.9	78.0	77.4	139.6	120.9	165.8	51.9			
(10; R = CH_3)	52.2	164.3	123.3	134.4	77.0	81.1	81.1	77.0	134.4	123.3	164.3	52.2
(11) †	4.6							108.3	146.7	62.3		
(11) [a]	3.9	79.2	65.2	69.2	59.4	67.5	74.9	107.8	149.7	62.6		
(12; R = CH_3) [b]	3.6	80.9	65.2	72.3	59.1	85.9	71.8	132.8	120.6	163.8	50.9	
(13; R = CH_3) †								136.0	123.3	166.6		

* In p.p.m. downfield from Me_4Si, as solutions in $CDCl_3$ and [a] CD_3OD or [b] C_6D_6. † Assignment of the remaining carbons was not possible due to the low solubility or instability of (11) in $CDCl_3$ and (13) in $CDCl_3$ or CD_3OD solvents.

It is interesting to compare these results with the data obtained in the present study. The extant data for the acetylenic derivatives (1)—(13) are summarised in the Table. The spectrum of dehydromatricaria ester [10] (12; R = CH_3) is also included for comparison. Several well established methods [11,12] common in ^{13}C spectroscopy were used in the assignment of the spectral peaks. Chemical shift theory associated with the multiplicity patterns obtained from off-resonance experiments and intensity data allowed the various types of carbon atoms to be readily differentiated. In addition, the combination of these methods also permits most types of *sp*-hybridised carbons to be easily assigned even for tri- or tetra-poly-ynes, *e.g.* dehydromatricaria ester.[10]

(1) $HC{\equiv}C{\cdot}CHO$
(2) $HC{\equiv}C{\cdot}CH_2OH$
(3) $HC{\equiv}C{\cdot}CH(OCH_2CH_3)_2$
(4) $HC{\equiv}C{\cdot}C{\equiv}N$
(5) $CH_3C{\equiv}C{\cdot}C{\equiv}CH$
(6) $CH_3C{\equiv}C{\cdot}C{\equiv}CCH_3$
(7) $HC{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CH_2OH$
(8) $HC{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CO_2CH_3$
(9) $N{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CO_2R$
(10) $RO_2C{\cdot}CH\overset{t}{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CO_2R$
(11) $CH_3C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CH_2OH$
(12) $CH_3C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CO_2R$
(13) $HOCH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CO_2R$

From the Table it is evident that the chemical shifts of the *sp*-hybridised carbons follow a regular pattern consistent with the nature of the substituent. When the substituent is the nitrile group the α-acetylenic carbon is shifted upfield by quite considerable amounts. Compared to the predicted value for acetylene (δ 72.8,[7] 73.9 [13]) or the observed values for disubstituted alkynes (*ca.* δ 75 [7]) this represents a shielding of *ca.* 17 p.p.m. On the other hand, the β-acetylenic carbon experiences only a small deshielding effect. This contrasts with the small

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α-effect which the nitrile group induces in saturated nitriles.[14] Since the carbon–carbon triple bond and the nitrile are both isoelectronic the large α-effect seen for the more polar group (17 compared to *ca.* 10 p.p.m. for the acetylenic group [7,9]) would suggest that inductive polarisation is significant in acetylenic nitriles. The considerable difference in chemical shift between the α- and β-acetylenic carbons in these compounds, which is a measure [8] of the extent of polarisation in the acetylenic linkage, also supports this conclusion. Although the α- and β-acetylenic carbons again experience contrary shielding effects in the compounds containing triple bonds directly linked to sp^2-hybridised carbons, the relative magnitudes of these effects are reversed. In this case it is the β-carbon which undergoes a large downfield shift (5—10 p.p.m.) whilst the sp^2-hybridised substituent induces only a small upfield shift of the α-carbon relative to the corresponding alkyl substituent. These results are thus in accord with a similar pattern earlier observed [9] with simple derivatives of alk-3-en-1-ynes.

The triple bond can make a significant contribution to the shieldings of its immediate α-carbon neighbours and can induce, in simple alkynes, diamagnetic shifts commonly of the order of *ca.* 10 p.p.m. In the present series of compounds this effect can also be seen operating. The chemical shift of the nitrile carbons which fall intermediate between those of simple alkynes and alkene and aromatic carbons reflect this shielding. Compared to saturated nitriles, which are observed [14] near δ 120, the acetylenic nitriles resonate at *ca.* 105 p.p.m. This is in good agreement with the value of 106.9 p.p.m. observed [15] for the nitrile carbon of phenylpropiolonitrile. Each of the other substituent α-carbons also experiences similar upfield shift due to the acetylenic group. Although the magnitude of these shifts varies irregularly, it would appear by comparison of the ^{13}C data for propynal (1) and propargyl alcohol (2) with the corresponding olefinic and alkyl compounds,[11] and the other compounds reported in this paper, that the upfield shift is generally greater for an sp^2- than for an sp^3-hybridised substituent. The larger shift effects evident for the sp^2-hybridised substituents presumably have their origin in polarisation of the triple bond through such forms as $-\overset{+}{C}=C=C-\overset{-}{X}$. Finally, it is interesting to note that the chemical shifts of olefinic carbons of the acetylenic esters (8)—(10), (12), and (13) follow a similar trend to that observed [16] in other αβ-unsaturated methyl esters allowing for the diamagnetic effect of the acetylene group on the β-carbon nucleus.

The ^{13}C n.m.r. spectra of the nitrile acid (9; R = H) and related compounds thus show that each carbon can be readily assigned and that funtionalised acetylenic compounds of this type follow the same additivity parameters as simple alkynes. Studies to further elaborate the α-effect of the acetylenic group and the application of this data to the biosynthesis of fungal metabolites of diatretyne 2 class are under investigation.

EXPERIMENTAL

The ^{13}C Fourier spectra were recorded on a Bruker HFX multi-nuclear spectrometer operating at 22.63 MHz. The compounds were measured as 25% (w/w) solutions in deuteriochloroform or indicated solvent containing *ca.* 5% tetramethyloilane (w/w). The data recorded are in p.p.m. downfield from the carbon resonances of the internal tetramethylsilane and are thought to be accurate to ±0.2 p.p.m. Compounds (1)—(8), (11), and (12) were prepared by well established methods whilst compounds (9), (10), and (13) were isolated from *L. glaucocana* by the reported procedure. All samples were purified immediately prior to use.

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