Tetrahedron Letters,Vol.25,No.3,pp 337-340,1984
Printed in Great Britain

0040-4039/84 $3.00 + .00

APPLICATION OF LONG RANGE J C-H RESOLVED 2D SPECTROSCOPY (LRJR) IN STRUCTURAL ELUCIDATION OF NATURAL PRODUCTS. THE STRUCTURE OF OXIRAPENTYN

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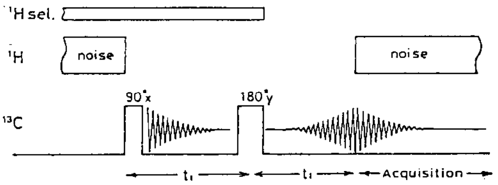
Summary: The structure of a fungal metabolite, oxyrapentyn has been determined as shown in Fig. 7 by application of a new NMR technique, long range J C-H resolved 2D spectroscopy (LRJR).

One of the problems in the structural elucidation of complicated molecules by NMR spectroscopy is how to connect proton spin systems separated by quaternary carbons or heteroatoms. This problem has been partly solved by ^{1}H-NMR NOE experiment and/or ^{13}C-{^{1}H} long range selective proton decoupling (LSPD)1). Although the latter method is very useful in case of aromatic or saturated systems, in which carbon signals to be observed are coupled to a few protons, it is not of use for compounds which give complicated splitting patterns of carbon resonances in the proton coupled ^{13}C-NMR spectra.

The technique proposed by Bax2), long range J C-H resolved 2D spectroscopy (LRJR), has overcome this problem and enables to observe selectively only long range couplings between carbons and a given proton. This method is based on an existing 2D-Fourier transform experiment3) which detects ^{13}C spin echoes modulated by heteronuclear spin-spin coupling, but it uses a frequency-selective 180° radiofrequency pulse applied to an isolated proton resonance. In our experiments, however, we used selective proton decoupling instead of the frequency-selective pulse because of experimental easiness, and the irradiation power was maintained at a very low level ($\gamma H_2/2\pi$= 11 Hz) so as to affect only the long range C-H coupling (Fig. 1). As pointed out by Bax2), the technique provides precise measurements of long range C-H couplings and therefore useful for studies of molecular conformation as evidenced in this work. We wish to report herein the application of LRJR for the structural determination of a fungal metabolite, oxyrapentyn4).

Fig. 1 pulse sequence for LRJR



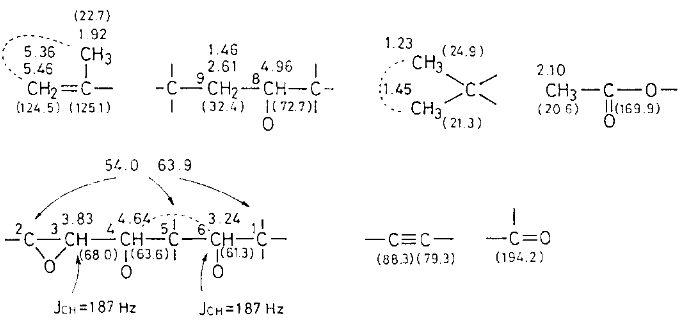
Oxyrapentyn (I) is an antibiotic produced by Beauveria felina SANK 13682 and active against gram positive bacteria4). The physicochemical properties of I is as follows; $C_{18}H_{20}O_6$ (EIMS, M$^+$ m/z 332, found C:65.42, H:6.05, calcd. C:65.05, H:6.07), mp. 114-115°C, $[\alpha]_D^{20}$ -111.7°(c 1, $CHCl_3$), UV λ_{max}^{MeOH} 225 nm ($E_{1cm}^{1\%}$480), IR ν_{max}^{KBr} 2230, 1740 and 1710 cm^{-1}.

The ^{13}C-NMR spectrum (100 MHz, $CDCl_3$)$^{5)}$ of I revealed the following carbons (in ppm); 4 X CH_3 (20.6, 21.3, 22.7 and 24.9), CH_2 (32.4), 4 X CH (61.3, 63.6, 68.0 and 72.7), CH_2= (124.5), 5 X -C- (54.0, 63.9, 74.7, 79.3 and 88.3), -C= (125.1), -O-C=O (169.9) and -C=O (194.2). Since the presence of a triple bond in I was suggested by the IR band at 2230 cm^{-1}, two quaternary carbon resonances at 79.3 and 88.3 ppm were ascribed to sp carbons. Two methine signals at 61.3 and 68.0 ppm were assigned to epoxide functions based on their large C-H coupling constants (J_{C-H}=187 Hz)$^{6)}$. Since the methine protons at 3.24 and 3.83 ppm appended to these carbons were not coupled to each other and no other carbons showed such large C-H coupling, it is evident that there exist two $-\overset{O}{CH-C}-$ moieties in I.

The 400 MHz ^1H-NMR$^{5)}$ ($CDCl_3$) of I showed the following signals (in ppm); 1.23 (CH_3, s), 1.45 (CH_3, s), 1.92 (CH_3-C=, dd, J=1.0 and 1.5 Hz), 2.10 (CH_3CO-, s), 5.36 and 5.46 (CH_2=C-, dq, J=1.5 and 1.5 Hz, dq, J=1.0 and 1.5 Hz), 3.24 (-CH-O-, bs), 3.84 (-C_3H-O-, d, $J_{3,4}$=2.0 Hz), 4.64 (-C_4H-O-, bd, $J_{3,4}$=2.0 Hz), 1.46, 2.61 (-HC_9H-, J_{gem}=15 Hz, $J_{9a,8}$=$J_{9b,8}$=3.0 Hz), and 4.96 (-C_8H-, t, $J_{8,9a}$=$J_{8,9b}$ =3.0 Hz).

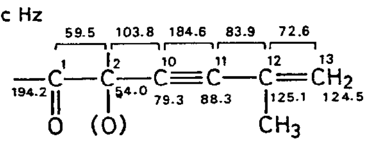
In the 2D-COSY spectrum of I, long range couplings were observed between methyl signals at 1.23 and 1.45 ppm; methine protons at 3.24 and 4.64 ppm; exomethylene protons (5.36 and 5.46 ppm) and an allyl methyl at 1.92 ppm. These results are summarized in the partial structures shown in Fig. 2 together with the C-H chemical shift correlation. No NOE effect observed between H_4 and H_6 excluded the arrangement of these two protons in an adjacent position with their dihedral angle being close to 90°. At this point, it is not certain which of C_1 and C_5 joins the epoxide formation with C_6.

Fig. 2



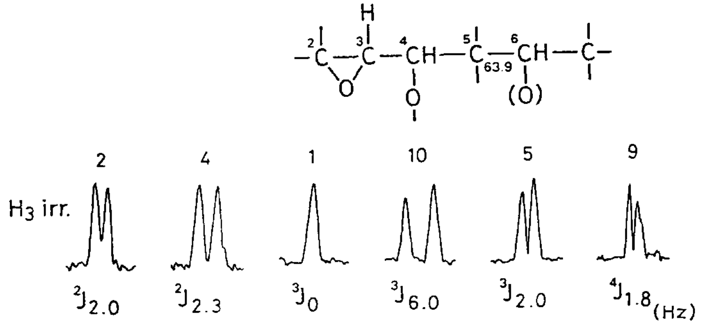
Since there exit many quaternary carbons in I, further structural information could not be obtained by ^1H-NMR spectral analysis. Thus, use was made of INADEQUATE technique$^{7)}$ which enables to detect selectively C-C spin coupling at natural abundance level. As a result, the partial structure containing C_1, C_2 and C_{10} to C_{13} was proved as shown in Fig. 3. However, this method was not so useful in the structural analysis of I, since it contains several kinds of C-C linkage including a triple bond and epoxides whose C-C coupling constants range from ca. 185 to 30 Hz. This situation required to repeat very time-consuming measurement several times by varying the time interval, τ, given by τ=1/4J_{C-C} for INADEQUATE experiment$^{7)}$. Therefore, we tried to apply LRJR$^{2)}$ for further structural analysis of I.

Fig. 3



Selective irradiation of H_3 caused C_2, C_4, C_5, C_9 and C_{10} to collapse to doublets (Fig. 4). Since C_2 and C_{10}, which are proved to be two or three bonds away from H_3 by their long range couplings ($^2J_{C-H}$ or $^3J_{C-H}$=2.0 and 6.0 Hz)$^{8)}$, are present in the partial structure (Fig. 3), C_3 must be connected to C_2 resulting in the epoxide ring formation. The spin coupling between C_5

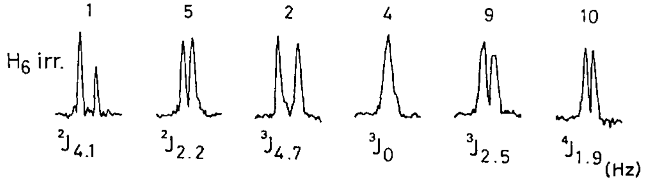
Fig. 4



and H_3 ($^3J_{C-H}$=2.0 Hz) connects C_3 and C_5 through C_4. The ^{13}C chemical shift of C_5 (63.9 ppm) supported its epoxide ring formation with C_6. The very small coupling constant between H_3 and C_1 ($^3J_{C-C}$=ca. 0 Hz) is explained in terms of their *trans* relationship with respect to the epoxide structure[8]. It is well known that the small coupling constants are observed with epoxide protons in the same stereochemistry[9]. This value is in sharp contrast to that of C_{10} which is in *cis* relationship to H_3. The coupling between C_9 and H_3 ($^4J_{C-H}$=1.8 Hz) is ascribed to W-form relationship of these atoms and therefore, C_9 must be connected to C_1 or C_5.

Irradiation of H_6 (Fig. 5) proved the couplings with C_1 (4.1 Hz) and C_2 (4.7Hz) which must be assigned to $^2J_{C-H}$ or $^3J_{C-H}$. Thus, C_6 and C_1 are directly connected to each other. The very small coupling between H_6 and C_4 (^3J=ca. 0 Hz) is explained by stereochemical relationship similar to that between H_3 and C_1 (*vide supra*). The magnitude of the coupling constant of C_9 (^3J=2.5 Hz) shows it to be linked to C_5. The coupling ($^4J_{C-H}$=1.9 Hz) between H_6 and C_{10} assignable to W-form relationship gave relative stereochemical information on the two epoxide rings (*vide infra*).

Fig. 5



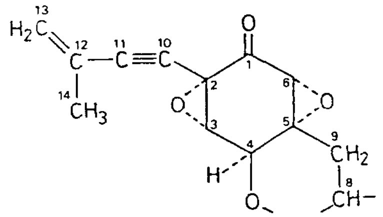


Fig. 6

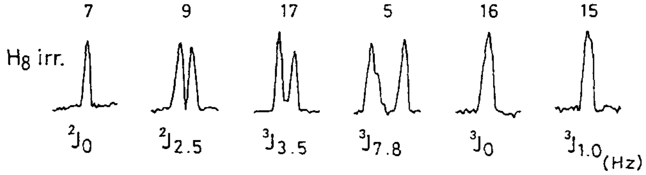
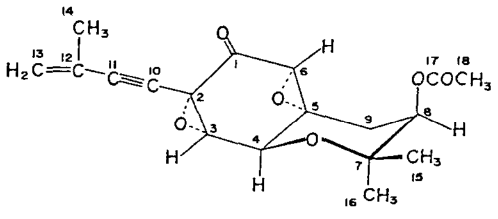


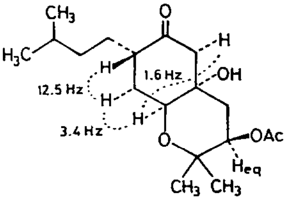
Fig. 7 The structure of oxyrapentyn



Irradiation of H_8 (Fig. 6) proved its coupling ($^3J_{C-H}$=3.5 Hz) with the ester carbonyl carbon, C_{17}, giving an evidence that the acetyl group is located on C_8. The only remaining function $(CH_3)_2C$- is thus connected to C_8 and the oxygen on C_4 to give the planar structure of I (Fig. 7). The very small or negligible C-H coupling constants observed between H_8 and geminal methyl carbons (C_{15} and C_{16}) suggest the equal dihedral angles between H_8-C_{15} and H_8-C_{16}, namely *gauche* relationship[10]. In this case, direct evidence for the linkage of C$_8$ and C$_7$ could not be obtained due to the lack of coupling between H$_8$ and C$_7$.

Further stereochemical information can be obtained by detailed analysis of H-H and C-H long range couplings[8)] (Fig. 7). The W-form relationships between H$_6$ and C$_{10}$ (^4J=1.9 Hz) and H$_3$ and C$_9$ (^4J=1.8 Hz) require planarity of these coupling pathways and therefore the two epoxide rings must be on the same side of the cyclohexane ring. The W-form long range coupling between H$_4$ and H$_6$ (J=1.5 Hz) defines the relative stereochemistry of H$_4$. The very large coupling constant between C$_5$ and H$_8$ ($^3J_{C-H}$=7.8 Hz) is explained by antiperiplanar relationship of these two atoms; thus, H$_8$ must be in an equatorial position. This geometry is in good agreement with the finding that H$_8$ is *gauche* to both the dimethyl carbons, C$_{15}$ and C$_{16}$ (*vide supra*). Thus the relative stereochemistry of I is determined as shown in Fig. 7. Hydrogenation of I with 10% Pd-C in MeOH gave a monodeoxydecahydro derivative, m.p. 148-149°C, C$_{18}$H$_{30}$O$_5$, EIMS m/z 326, which was assumed to possess the structure as shown in Fig. 8 by NMR spectral analysis. The CD spectrum of the compound showed a negative Cotton effect ([Θ] -1865 , at 294 nm). Therefore, the absolute stereochemistry of oxyrapentyn has been determined as shown in Fig. 7.

Fig. 8



It should be emphasized that LRJR afford an excellent methods for observing C-H long range coupling and therefore will become a very important technique for structural elucidation of natural products.

Acknowledgement This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (No. 57560117 to H.S.).

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(Received in Japan 10 October 1983)