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NATURAL ABUNDANCE $^{13}C-^{13}C$ COUPLING CONSTANTS OBSERVED VIA DOUBLE QUANTUM COHERENCE. THE TWO-DIMENSIONAL 'INADEQUATE' EXPERIMENT FOR ^{13}C NMR SPECTRAL ANALYSIS OF ERYTHRONOLIDE - B

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Summary A one- and a two-dimensional INADEQUATE NMR experiment afforded one-bond $^{13}C-^{13}C$ coupling constants at natural abundance and total unambiguous signal assignment for erythronolide-B (1).

In view of their medical importance, the 14-membered macrolide antibiotics have been extensively studied by ^1H n m.r spectroscopy [1]. Conformational informations of considerable value based on angle-dependent long-range $^{13}C-^{13}C$ and $^{13}C-^1H$ coupling constants [2] would be expected to complete previous ^1H n m r results However, these investigations require precise carbon-13 n m r signal assignment in the spectra of these antibiotics Earlier spectral data were not totally unambiguous [3]. The aglycone of most of the 14-membered macrolides are biogenetically derived exclusively from propionate units [4] Thus incorporation of a biosynthetic precursor is not of a great help in such studies because several carbon atoms of close chemical shift are simultaneously labeled [5].

In this letter we report one-bond $^{13}C-^{13}C$ coupling constants and a total and unambiguous assignment of the carbon-13 n m.r spectrum of erythronolide-B (1) (Table). The results obtained with the new one- and two-dimensional INADEQUATE n m.r experiments [6] may form the basis of any further investigation aimed at evaluating long-range $^{13}C-^{13}C$ or $^{13}C-^1H$ coupling constants on (1)

Carbon-13 n.m.r spectral analysis of (1) was attempted at 100.62 MHz with a Bruker WM-400 spectrometer. The double quantum ^{13}C n.m r. pulse sequence was used [6a]. This allowed the detection of carbon-carbon coupling constants at natural abundance with suppression of the strong signals from molecules with a single carbon-13 nucleus [6] (Figure). A solution of (1) was prepared in pyridine -d_5 (800mg in 1 8 ml) and the INADEQUATE pulse sequence was optimized for $^1J_{CC}$ 40Hz ($J\tau=1/4$), data were accumulated at 85°C for 12 h. The number of data points was 16000 with a frequency range of 8770 Hz giving a digital resolution of 1 1 Hz/point

An unambiguous analysis of this one-dimensional INADEQUATE spectrum was not possible as a result of a number of carbon-carbon coupling constants of very close value As a consequence , a two-dimensional INADEQUATE study was undertaken

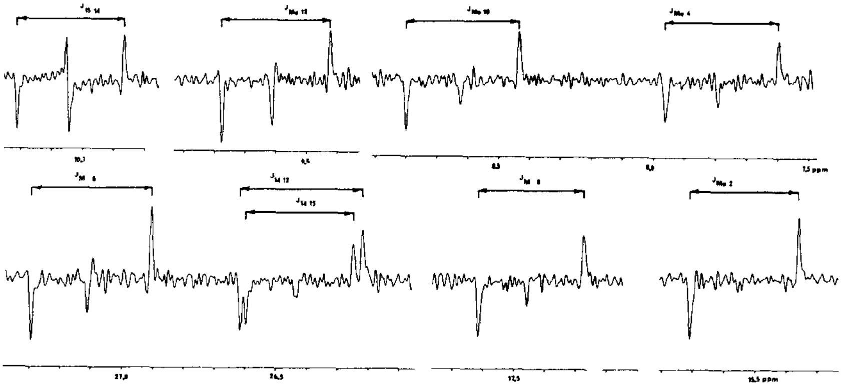


Figure The high field part (from 7.5 to 27.3 ppm) of the 100.62 MHz 1D-INADEQUATE ^{13}C n m r spectrum of (1) recorded in pyridine-d_5 at 85°C Maximum signal intensity has been selected for J_{CC} = 40 Hz ($J\tau$=1/4).

on (1) as described by Freeman [6b] The two-dimensional spectrum was recorded at 75°C using 600mg of (1) in 1.8 ml pyridine-d_5. The data optimized for maximum signal intensity with $^1J_{CC}$ 40 Hz were accumulated overnight , quadrature detection in both dimensions was employed. The spectral width for F_1 was $\pm$ 8770 Hz and for F_2 was 8770 Hz Directly coupled carbon-13 resonances were idendified by the fact that they generate the same double quantum frequency and thus appear in one row of the two-dimensional data matrix This technique permitted the determination of the carbon connectivities in the right-half (from C-2Me/C-2 to C-8/C-8Me) and in the left-half (from C-10Me/C-10 to C-14 /C-15of erythronolide-B (1) and allowed a precise carbon signal assignment to be carried out for this molecule (Table). Satellite signals due to the two-bond coupling **constant**($^2J_{8,10}$ $\sim$10.8 Hz) through the C-9 ketone appear in the 1D-INADEQUATE spectrum of (1)[7] However, the intensity of these two-bond satellites is strongly reduced because of the optimization value of 40 Hz and also as a result of the strong AB system (C-8/C-10 $\Delta\nu$ 59Hz)[8]. Thus the two-dimensional spectrum of (1) was of indispensable help for evaluating the one-bond ^{13}C-^{13}C coupling constants of erythronolide-B (1) (Table).

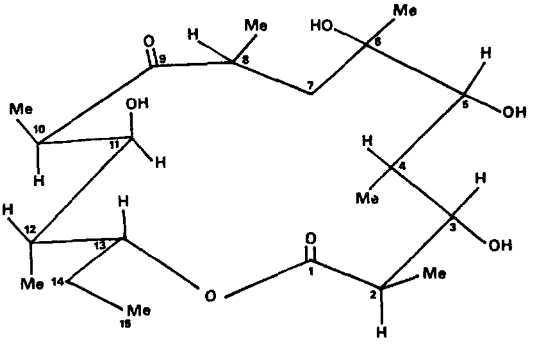
As expected, the one-bond coupling constants ($^1J_{CC}$) cannot be correlated with the equatorial- and axial-like configuration of the substituents on the diamond-lattice type conformation of erythronolide-B (1) [1] The relatively low value of the coupling constants $J_{2,3}$ and $J_{10,11}$ reflect the α,β-position of these linkages with respect to a carbonyl group Such an effect has been demonstrated previously on cyclohexanone systems [7a] . Interestingly , $J_{7,8}$ does not show the influence of the C-9 ketone in the same way, this coupling constant is normal ,considering sp^3 hybridized carbon atoms not attached to hete-

TABLE

^{13}C n.m.r. spectral data for erythronolide-B (1)[a]

carbon	chemical shift ppm[b]	coupled with carbon	one-bond coupling constant (Hz)
C-1	d		
C-2	44 88	C-3	36 75
C-3	79.91	C-4	38.00
C-4	37.39		
C-5	82 03	C-4	38.75
		C-6	39.25
C-6	75.84		
C-7	40.34	C-6	39.50
		C-8	33.50
C-8	42 91		
C-9	d		
C-10	42.32	C-11	36.75
C-11	71.01		
C-12	41.20	C-11	39.25
		C-13	39 25
C-13	75.51		
C-14	26.47	C-13	40.00
C-15	10.81	C-14	35.00
C-2Me	15 64	C-2	35 50
C-4Me	7.92	C-4	36.75
C-6Me	27.15	C-6	39.00
C-8Me	17.54	C-8	34 50
C-10Me	9 82	C-10	36 75
C-12Me	9.67	C-12	35.50

[a] the corresponding numbering scheme is given with the structural formula , [b] chemical shifts were measured with respect to pyridine-d_5 (149 9 ppm) and are given for TMS = 0 , [c] coupling constants are shown only once for all coupling pairs at that nucleus where the J values could be the more precisely determined One-bond coupling constants for the following linkages were not measured C-1/C-2 , C-8/C-9 and C-9/C-10 ; [d] these chemical shifts were not measured



ERYTHRONOLIDE - B (1)

roatoms[9] The value of $J_{13\,14}$ is higher than any other coupling constant due to oxygen-bonded carbon-carbon linkages This result is interpreted by the fact that $J_{13,14}$ is not part of the 14-membered macrolide ring In a general study on the one-bond carbon-carbon coupling constants of hexopyranosides[10], it has been shown recently that the highest $^1J_{CC}$ value (besides $J_{1,2}$) corresponds to the C-5/C-6 linkage

As far as the C-methyl involved bonds are concerned, the coupling constants exhibit values from 34.50 to 39.00 Hz (Table). The relatively great variation of $^1J_{CC}$ helps in the determination of matching satellite pairs. However, no interpretation based on the conformation of the molecule can be advanced at the moment to understand the size of each of these coupling constants

In the light of the results presented in this letter, previous signal assignments for C-7, C-10, C-8Me and C-10Me of erythronolide-B(1) measured in dioxan-d_8 should be revised[3a] although without serious consequences for the interpretation of the labeling pattern in a biosynthetic experiment[11] Our assignment of C-14 and C-6Me is also different from earlier data, however, this may be due only to a solvent effect

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