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NATURAL ABUNDANCE ^{13}C - ^{13}C COUPLING CONSTANTS OBSERVED VIA DOUBLE QUANTUM COHERENCE.STRUCTURAL ELUCIDATION BY THE ONE- AND THE TWO-DIMENSIONAL NMR EXPERIMENTS OF VELLOZIOLONE , A NEW SECO-DITERPENE.

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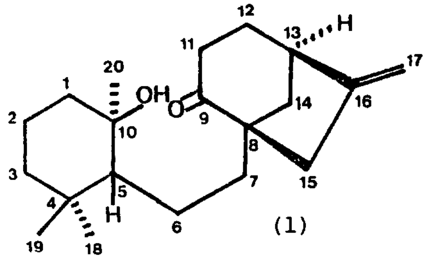
Summary : With the help of Natural Abundance ^{13}C - ^{13}C Coupling Constants , observed via double quantum coherence , the structural determination of VELLOZIOLONE (1) , a new diterpene , has been carried out.

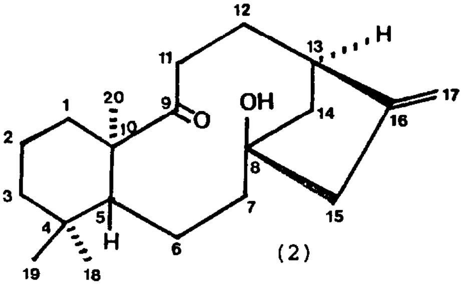
During the course of a continuing phytochemical survey of Brazilian Velloziaceae , we have examined a sample of Vellozia caput-adeae L.B. Smith & Ayensu , a species occuring in Diamantina , State of Minas Gerais , Brazil , from which a new diterpene , velloziolone (1) was isolated.With the help of 'INADEQUATE' n.m.r. experiments [1] we establish herein its structure.To our knowledge , this example is the first application of the two-dimensional Freeman technique[1b] for studying a naturally occurring substance of unknown constitution.

The molecular formula of velloziolone (1) , ($C_{20}H_{32}O_2$), m.p. 113-114°C $[\alpha]^{\lambda}_{24°}$ $\overset{589}{-58.5}$ $\overset{578}{-60.8}$ $\overset{546}{-70.8}$ $\overset{436}{-135.2}$ $\overset{365}{-276.9}$ (c 0.83 , $CHCl_3$) , was determined by microanalysis and by mass spectrometry [m/e 304.2377 ($M^{+\cdot}$)] .Its 100 MHz ^1H n.m.r. spectrum ($CDCl_3$) showed the folloing clearly resolved signals : three methyl singlets at 0.75 , 0.92 and 1.15 ppm and two olefinic protons at 4.92 and 5.02 ppm.Based on conventional carbon-13 n.m.r. data (Table) , on the results given above and on the structure of other compounds isolated from Vellozia species [2] , two different seco-ent-kauren-16-eno structures (1) and (2) were considered as a working hypothesis for the new diterpene.

Carbon-13 n.m.r. spectral analysis of velloziolone was first attempted at 50.31 MHz with a Bruker WM-200 spectrometer.The double quantum pulse sequence was used [1a].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.Although , a number of matching satellite pairs were identified from this experiment , the structure of velloziolone (1) could not be established.Ambiguities were due to the close chemical shift of some resonances (Table) and especially to coupled nuclei forming strong AB systems with reduced satellite intensities [3].Therefore , the spectral study was attempted again at 100.62 MHz with a Bruker WM-400 spectrometer. A solution of velloziolone (1) was prepared in dioxan-d_8 [850 mg of (1) in 1 ml solvent] .As a preliminary experiment , first an APT-type spectrum was measured [4].This defines the multiplicity



(1)



(2)

(number of bound protons) for each carbon signal and allows the determination of the maximum number of carbon connectivities possible for each carbon atom.Then , the double quantum pulse sequence was optimized for $^1J_{CC}$ = 44 Hz ($J\tau$ = 1/4) in order to observe coupling constants for both sp^3-sp^3 and sp^2-sp^3 type linkages with the maximum intensity in one experiment.Data was accumulated at 95°C overnight using a recycle time of 4.33 sec.The number of data points was 16K with a frequency range of 6040 Hz giving a digital resolution of 0.75 Hz/point.Resolution enhancement with the Gaussian multiplication technique was applied [5].

Analysis of the results permitted to recognize 17 one-bond and 1 two-bond matching satellite pairs (Table and Figure).Although , this 1D-INADEQUATE experiment suggested structure (1) for velloziolone , 5 one-bond coupling constants ($J_{8,9}$, $J_{11,9}$, $J_{13,16}$, $J_{15,16}$ and $J_{16,17}$) were missing from the spectrum based on formula (1).A choice between structures (1) and (2) was extremely simple with the help of this powerful new n.m.r. technique.For instance the latter structure would imply the 73.69 ppm carbon signal to be coupled with three methylene type carbon resonances.However , inspection of the spectral data indicates clearly that the neighbours of the 73.69 ppm carbon signal are a methine , a methylene and a methyl type resonance , in agreement with structure (1).

Missing satellites were due to carbon atoms involved in bonds with each of the two carbon atoms C-9 and C-16 , expected to have especially long relaxation times.It has been shown previously that couplings to carbons exhibiting long T_1 values were suppressed or have given strongly reduced satellites in intensity if the recycle time used was too short [2b,6].However, unambiguous evidence was afforded for the connectivity between C-8/C-9 and C-9/C-11 by the detection of the two-bond coupling constant $J_{8,11}$ (Table).Such coupling constants ($^2J_{CC}$) through a ketone have been shown to be of the order of 9 - 12 Hz in cyclohexanone systems [6]. The 1D-INADEQUATE spectral analysis of terpenic molecules at high magnetic field is often very difficult.Complications are due to the similarity of most coupling constants and to the rather important isotope shifts.Furthermore , the coupling carbon pairs display AX or only weak AB systems.

In order to establish the complete connectivity of the molecular framework of velloziolone and to confirm the 1D-n.m.r. results , a two-dimensional study was undertaken on (1) as described by Freeman [1b].The two-dimensional spectrum was recorded on the sample used in the 1D-INADEQUATE experiment , at 60°C in order to shorten spin-lattice relaxation times.The data, TABLE

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

carbon	chemical shift [b] 95°C in dioxan-d_8	chemical shift [b] 25°C in $CDCl_3$	coupled with carbon [c]	one-bond coupling constant (Hz)
C-1	43.71	42.5	C-2	32.5
			C-10	37.8
C-2	20.99	20.3	C-3	32.8
C-3	42.40	42.1	C-4	32.3
C-4	35.91	35.2		
C-5	58.64	57.9	C-4	33.3
C-6	22.79	21.9	C-5	36.0
C-7	38.48	38.1	C-6	35.0
C-8	57.93	57.4	C-7	38.5
			C-9	not measured
			C-14	28.5
			C-15	32.3
C-9	211.93	213.8		
C-10	73.69	73.9	C-5	37.0
C-11	36.14	35.8	C-9	not measured
			C-12	31.8
C-12	34.07	33.6		
C-13	43.25	42.3	C-12	31.5
			C-16	not measured
C-14	43.84	42.7	C-13	32.5
C-15	42.38	41.4	C-16	not measured
C-16	154.17	152.2	C-17	not measured
C-17	106.28	106.4		
C-18	22.07	21.2	C-4	35.8
C-19	33.19	32.6	C-4	36.3
C-20	24.18	23.5	C-10	39.5

[a] the corresponding numbering scheme is given with the structural formula; [b] chemical shifts were measured in the conventional experiment (in $CDCl_3$) with respect to internal TMS and in the INADEQUATE experiment with respect to dioxan-d_8 (66.5 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. A two-bond coupling constant of 9.3 Hz was measured for $J_{8,11}$.

optimized for maximum signal intensity with $^1J_{CC}$ = 44 Hz , were accumulated overnight. Quadrature detection in both directions and a relaxation delay of 2.0 sec were employed. The spectral width for F_1 was ± 17241 Hz and for F_2 was 20833 Hz. Directly coupled carbon-13 resonances were identified by the fact that they generate the same double quantum frequency and thus appear in one row of the two-dimensional data matrix.

In addition to informations obtained by the 1D-experiment , the 2D-INADEQUATE spectrum has permitted to determine the relationship of C-16 with respect to its neighbours. However, the direct evidence for the connectivity of C-8/C-9 was not obtained as both of these quaternary carbons have long T_1 values. The axial configuration of C-20 of (1) is obvious (23.5

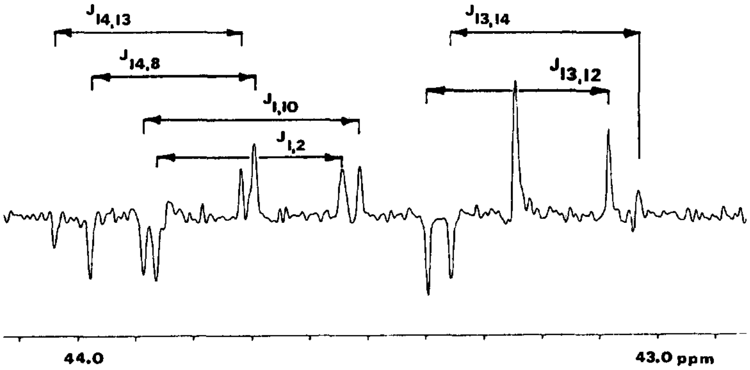


Figure.Section of C-13 , C-1 and C-14 from the 100.62 MHz INADEQUATE ^{13}C n.m.r. spectrum of (1) recorded in dioxan-d_8 at 95°C.Maximum signal intensity has been selected for J_{CC}=44 Hz ($J\tau$ 1/4).Satellites due to $J_{13,16}$ cannot be clearly detected because of the long T_1 value of C-16.Satellites due to $J_{13,14}$ and $J_{14,13}$ exhibit a typical strong AB system ($J/\Delta\nu$ 0.55); at 50.31 MHz this AB system ($J/\Delta\nu$ 1.1) could not be observed [3].

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ppm in $CDCl_3$) [7].Chemical shifts of C-5 , C-8 and C-13 are in agreement [7] with the stereochemistry shown which is the same in previously isolated related compounds [2a].

The digital resolution in this 2D-study was insufficient to determine precise coupling constants.The latter (Table and Figure) result from the 1D-INADEQUATE experiment described above.It is worth noting that the low values of the coupling constants (Table) corresponding to $J_{8,14}$ and $J_{11,12}$ reflect the α ,β position of these linkages with respect to the C-9 ketone.Such an effect has been demonstrated previously on cyclohexanone systems [6].

This investigation illustrates the advantages of high magnetic field for structural studies based on natural abundance ^{13}C - ^{13}C coupling constants.The study could be completed in spite of the unfavourable dispersion of the carbon signals , five of which appear in (1) within 1.46 ppm in the 42 - 44 ppm region.

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