Natural Abundance ^{13}C–^{13}C Coupling Constants observed *via* Double Quantum Coherence: Structural Elucidation of Velloziolide, a Diterpene with a Novel Skeleton

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A diterpene with a novel skeleton, velloziolide, has been isolated from *Vellozia Candida* Mikan; its structure has been established mainly by using natural abundance ^{13}C–^{13}C coupling constants observed *via* double quantum coherence.

Continuing our work on the constituents of the Brazilian plant *Vellozia Candida* Mikan, we have recently isolated a diterpenic lactone with a novel skeleton, designated velloziolide (1), and report herein its structural elucidation. To our knowledge this is the first example of the application of natural abundance ^{13}C–^{13}C coupling constants observed *via* double quantum coherence[1] to the structural determination of a natural product.

The molecular formula of velloziolide (1), m.p. 138—140 °C, $[\alpha]_D^{22}$ −13° (c 1, CHCl$_3$), C$_{20}$H$_{26}$O$_3$, was established by microanalysis and by mass spectrometry [m/e 314.1859 (M$^+$·)]. Its 400 MHz ^{1}H n.m.r. spectrum (CDCl$_3$) showed signals δ 1.22 (s, 3H, H-17), 1.42 (s, 6H, H-18 and H-19), 1.62 and 1.77 (2m, 2 × 2H, H-2 and H-3), 2.10 (s, 3H, H-20), 2.26 and 2.46 [d (AB), 2H, J 12.5 Hz, H-12 or H-14], 2.56 (t, 2H, $J_{1a,1e} = J_{1a,2a}$ 7 Hz, H-1), 2.66 and 2.78 [d (AB), 2H, J 13 Hz, H-14 or H-12], 5.05 and 5.10 (2d, 2 × 1H, $J_{16,15}$ 11.0 Hz and $J_{16',15}$ 17.0 Hz, H-16), 5.80 (s, 1H, exchangeable with D$_2$O, OH), and 5.92 (dd, 1H, $J_{15,16}$ 11.0 Hz and $J_{15,16'}$ 17.0 Hz, H-15).

The structure of (1), except for the absolute configuration at C-13, was established on the basis of mass, ^{1}H n.m.r., and ^{13}C n.m.r. spectroscopic data. Carbon-13 n.m.r. spectral analysis of (1) was attempted at 100.61 MHz with a Bruker WM-400 spectrometer using Freeman's 'INADEQUATE' technique[1] (Figure 1). This enabled us to determine carbon–carbon couplings at natural abundance by suppressing the strong signals from molecules with a single ^{13}C nucleus. A saturated solution of (1) was prepared in boiling [^{2}H$_8$]-dioxan [500 mg of (1) in 1 ml of solvent] and the spectrum, optimized for the 'INADEQUATE' experiment at $J = 60$ Hz ($J\tau = 1/4$),[1] was accumulated at 95 °C for 18 h. Detection of the satellite signals required a 4 s delay time and extensive accumulation because of the long relaxation times of the neighbouring quaternary aromatic carbon atoms of (1).

Optimization of the spectrum at $J = 60$ Hz ($J\tau = 1/4$)[1] permitted the observation of 15 satellite pairs due to one-
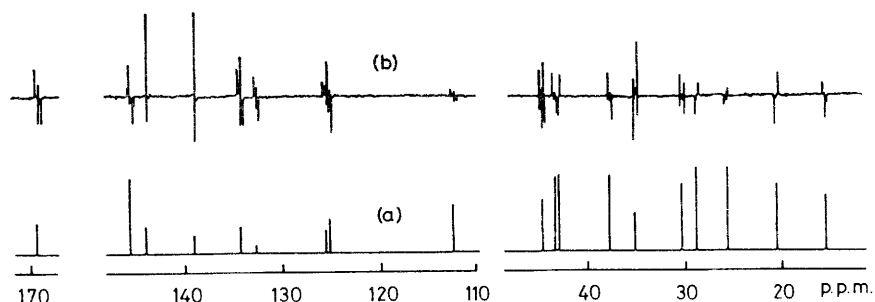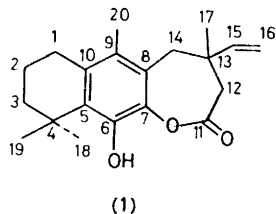

(1)

bond ^{13}C–^{13}C coupling constants. However, in view of the molecular formula of (1) it was clear that some carbon–carbon coupling constants were missing from the 'INADEQUATE' spectrum. Such situations prevail for aromatic carbon atoms where both sites involved in the coupling exhibit long relaxation times. Furthermore, missing satellite signals were expected for carbon atoms involved in strong AB systems ($J/\Delta v > 0.25$).[2]

The expected number of ^{13}C–^{13}C coupling constants for each carbon signal was easily deduced from chemical shift information and from a polarisation transfer spectrum via the INEPT technique[3] enabling an unambiguous determination of the number of hydrogen atoms borne by the various carbon atoms of (1). As a result of the optimization at $J = 60$ Hz the satellite lines outside the frequency range of 40—60 Hz, due to $J_{15,16}$ (70 Hz), as well as those due to couplings between sp^3 hybridized atoms (ca. 33.5 Hz)[4] appeared with low intensity (Figure 2). In contrast with the satellite lines of high amplitude for $J_{11,12}$ (54.9 Hz), signals representing ^{13}C–^{13}C couplings between substituted aromatic carbon sites had considerably reduced intensities. Thus the satellite lines due to $J_{9,10}$ (57.7 Hz) were small and those corresponding to $J_{5,6}$ and $J_{7,8}$ were hidden in the spectral noise. The reason why the $J_{6,7}$ coupling was missing in the spectrum of (1) under the experimental conditions used[1] was interpreted in terms of a large ^1J value of 76—78 Hz[4] and the known relationship between the intensity of the signal and the size of the coupling constant.[1] The absence of satellite lines for the three other carbon–carbon couplings between C-5/C-10, C-8/C-9, and C-12/C-13 was understood[2] to be a consequence of the strong AB systems corresponding to the following calculated $J/\Delta v$ values[1] of 0.36, 1.93, and 0.25 respectively.

Thus, matching satellite pairs and the expected absence of some satellite signals due to strong AB systems furnished unambiguous proof (Table 1) for the carbon connectivity pattern of (1) except for the disposition of the substituents on C-6, C-7, and C-8. ^{1}H–^{1}H coupling constants and ^{13}C n.m.r.[5] chemical shift considerations were in complete agree-

Figure 1. (a) The proton-decoupled ^{13}C n.m.r. spectrum of velloziolide (1) at 100.61 MHz and (b) the same spectrum observed via double quantum coherence.[1]

Figure 2. Section of (a) C-15 and (b) C-14 signals from the 100.61 MHz 'INADEQUATE' ^{13}C n.m.r. spectrum of (1). Optimization at $J = 60$ Hz ($J\tau = 1/4$); satellites due to small ($J_{14,13}$ 33.2 Hz) and to large ($J_{15,16}$ 70.0 Hz) coupling constants appear with reduced intensity and in the case of $J_{14,13}$ with opposite sign.[2]

Table 1. ^{13}C N.m.r. spectral data for velloziolide (1).

Carbon	Chemical shift[a]/p.p.m. (Me$_4$Si = 0)	Coupled with carbon	One-bond coupling constant/Hz
C-1	30.1	C-2	33.4
C-2	20.3	C-3	33.0
C-3	42.6	C-4	33.0
C-4	34.9		
C-5	132.5	C-4	42.8
C-6	143.9		
C-7	138.9		
C-8	125.1	C-14	43.6
C-9	125.4	C-20	46.5
C-10	134.1	C-1	42.5
		C-9	57.7
C-11	169.2	C-12	54.9
C-12	42.9		
C-13	44.2		
C-14	37.5	C-13	33.2
C-15	145.5	C-13	43.2
		C-16	70.0
C-16	112.3		
C-17	28.6	C-13	35.2
C-18	28.6	C-4	35.2
C-19	25.4	C-4	35.2
C-20	15.3		

[a] Chemical shifts were measured with respect to [^{2}H$_8$]dioxan (66.5 p.p.m.) and are given for Me$_4$Si = 0. The one-bond coupling constants are shown only once for all coupling pairs at that nucleus where the ^1J values could be determined more precisely. Satellite signals for the following linkages could not be observed (see text): C-5/C-6, C-6/C-7, C-7/C-8, C-5/C-10, C-8/C-9, and C-12/C-13.
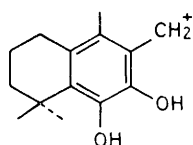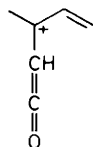
m/e 219   m/e 95

ment with the carbon signal assignments determined on the basis of the measured ^{13}C–^{13}C coupling constants. From the observed ^{13}C chemical shifts it was obvious that two oxygen atoms of (1) were in an *ortho*-relationship.[5] The attachment of the phenolic hydroxy-group to C-6 was established by the induced downfield shift of 0.38 p.p.m. for C-18 and C-19 when the ^{1}H n.m.r. spectrum of (1) was measured in [^{2}H$_5$]-pyridine solution.[6] Location of the hydroxy-group at C-6 was also in agreement with the deuterium-induced isotope shifts observed for the *ortho*-carbon atoms in the conventional ^{13}C n.m.r. spectrum of (1) in which the phenolic hydrogen atom was exchanged for deuterium. Further evidence for the structure of velloziolide (1) is afforded from the ions *m/e* 219 and *m/e* 95 of its mass spectrum. These ions are readily rationalized in terms of structure (1).

As this example illustrates, the determination of the one-bond ^{13}C–^{13}C coupling constants is extremely useful for the establishment of the carbon connectivity pattern of unknown organic molecules.

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