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Note

Total NMR chemical shift assignments of baccharis oxide via 2D-INADEQUATE

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ABSTRACT: The ^{1}H and ^{13}C NMR spectra of baccharis oxide, a common triterpene in many *Baccharis* species, were assigned unambiguously by C–C correlations (2D-INADEQUATE). © 1998 John Wiley & Sons Ltd.

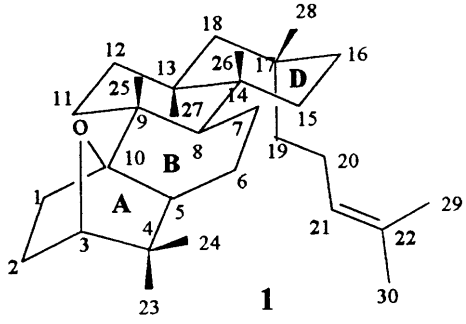
KEYWORDS: baccharis oxide; NMR; ^{1}H NMR; ^{13}C NMR; 2D-INADEQUATE

INTRODUCTION

Baccharis oxide (**1**) has been isolated from several *Baccharis* species studied by our group[1] and the structure of this unusual triterpene was elucidated by Mo *et al.*[2] based on x-ray diffraction experiments. However, no complete assignment of the NMR signals has been published. This prompted us to obtain the complete and unambiguous ^{1}H and ^{13}C NMR chemical shifts assignments using 2D-INADEQUATE to establish the C–C correlations while ^{1}H NMR, HETCOR and COLOC provided the assignment of all hydrogen signals.

RESULTS AND DISCUSSION

From decoupled ^{13}C NMR, DEPT-90 and DEPT 135 measurements we could separate the signals into four groups: eight methyls (δ 15.4, 17.5, 20.0, 22.0, 22.8, 24.2, 25.7, 32.8), 11 methylenes (δ 19.6, 20.1, 23.0, 24.8, 29.4, 29.8, 32.0, 32.3, 34.6, 43.0, 44.2), four methines (δ 39.1, 53.1, 84.3, 125.3) and seven quaternary carbons (δ 31.8, 36.5, 37.3, 39.1, 43.3, 93.8, 130.8). Further attempts to assign the signals using H–C one-bond (HETCOR) and multiple-bond (COLOC) correlations led to ambiguous assignments owing to the lack of proper models in the literature. We therefore focused our ^{13}C NMR signal assignment on the C–C correlations (2D-INADEQUATE). The expanded contour plot of the 2D-INADEQUATE experiment for baccharis oxide (**1**) is given in Fig. 1; ^{13}C pairs are circled and numbered according to their connectivities. The experiment was optimized for sp^3–sp^3 connectivity. Addition of $Cr(acac)_3$ allowed us to use a shorter recycle delay. All connectivities were found.



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The complete carbon sequence was obtained by assigning the signal at δ 93.8 to C-10 as the starting point. This carbon correlated with signals at δ 53.1 (C-5), 32.0 (C-1) and 37.3 (C-9). From C-1 we could obtain the chemical shift of C-2 at δ 24.8, which in turn was correlated with the signal at δ 84.3 (C-3), and the latter showed a correlation with the signal at δ 43.3 (C-4). The total assignment of ring A was obtained when the correlation between the signal at δ 43.3 (C-4) and 53.1 (C-5) was detected. Using the same approach with C-9, C-13 and C-17, we could accomplish the total assignment depicted in Table 1. From these data, the hydrogen assignments were obtained from the H–C correlations (Table 1).

Although the assignment of all NMR signals was unambiguous, we were intrigued by the deshielding of the axial H-11 chemical shift (δ 2.23, td, $J = 14$). A Dreiding molecular model of the molecule was scrutinized looking for a plausible explanation. The electron pairs of the oxygen belonging to ring A might be responsible for this deshielding but a change in the conformation of ring C (from chair to boat) would certainly locate the β-hydrogen at position 11 under the spatial influence of the two lone pairs of electrons of the oxygen, thus explaining the deshielding. An NOE difference experiment where the two hydrogens at δ 2.23 was irradiated did not increase the signal at δ 1.23 (Me-25). From this result, we suggest (although conscious that the absence of an NOE effect is not always conclusive[3]) that the conformation of ring C is chair as depicted in

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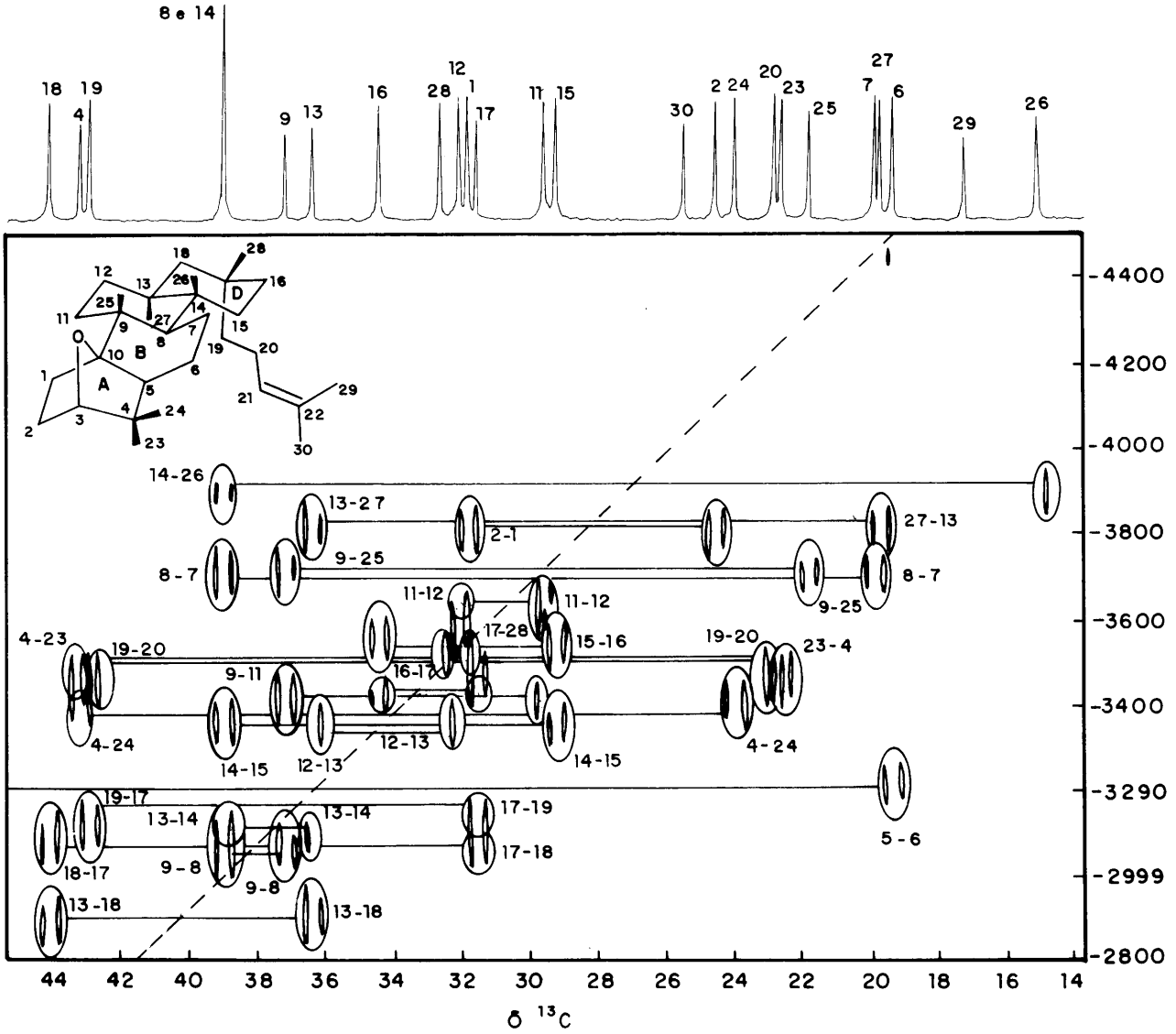

Figure 1. Expansion of the 75.5 MHz ^{13}C NMR 2D-INADEQUATE contour plot of **1** (0.25 M **1** in $CDCl_3$) with 0.03 M $Cr(acac)_3$ added. The 1D ^{13}C NMR spectrum (partial) is shown above with the signal assignment.

Fig. 1, which is also the conformation observed in the solid state through x-ray diffraction.[2]

In conclusion, a combination of 1D and 2D NMR techniques allowed the unambiguous assignment of all carbon and hydrogen of baccharis oxide. We were also able to determine the conformation of ring C in solution.

EXPERIMENTAL

The wetting point was recorded with a Kofler hot-plate set-up with a Thermopan microscope (C. Reichert Optische Werke). The optical rotation angle was measured in a Carl Zeiss Polamat A Rutina polarimeter.

^1H NMR spectra were recorded with a GEMINI 300 (300.1 MHz, Varian) or Bruker AC 300P spectrometer. $CDCl_3$ was used as the solvent, with Me_4Si (TMS) as internal standard. ^{13}C NMR spectra were obtained with a GEMINI 300 (75.5 MHz, Varian) or a Bruker AC 300P spectrometer. $CDCl_3$ (77.0 ppm) was used as internal standard.

The number of hydrogens attached to the carbon atoms were obtained from the DEPT-135 spectra (distortionless enhancement by polarization transfer) and DEPT-90 spectra. The ^{13}C 2D INADEQUATE spectrum was recorded on the Bruker AC 300P instrument operating at 75.5 MHz. (106 mg of 1 in 0.9 ml of $CDCl_3$, 5 mm tube, 298 K). $Cr(acac)_3$) (about 10 mg, 0.03 M) was added to shorten the relaxation times to about 1 s. The pulse sequence of Mareci and Freeman[4] was available on the Bruker spectrometer; 1024 scans were accumulated for each of 96 FIDs of 4K data points. Recycle delays of 2 s and a value of $\tau = 6.25$ ms were taken in the sequence $[\tau = (2n + 1)/4J(cc), n = 0]$. Gaussian multiplication was applied to both dimensions. The total acquisition time was 60 h.

Baccharis oxide, m.p. = 147.0–149.3° C and $[\alpha]_D^{25} = +39°$ (c 1.0, $CHCl_3$), was present in the hexane extract

Table 1. ^{1}H and ^{13}C NMR chemical shift data for baccharis oxide

Carbon	$\delta\,^{13}C$ (ppm)	^{13}C–^{13}C correlations (2D-INADEQUATE)	$\delta\,^{1}H$ (H–C correlation, HETCOR)
C-1	32.0	93.8 × 24.8	1.94 (overlap of signals, H_{exo}–C-1)
		C-10 × C-2	1.43 (m, H_{endo}-C-1)
C-2	24.8	84.3 × 32.0	1.90 (overlap of signals, H_{exo}–C-2)
		C-3 × C-1	1.67 (overlapped by the methyl signal, H_{endo}–C-2)
C-3	84.3	43.3 × 24.8 (C-4 × C-2)	3.75 (d 1, J = 5.7 Hz, H–C-3)
C-4	43.3	84.3 × 53.1 × 22.8 × 24.2	
		C-3 × C-5 × C-23 × C-24	
C-5	53.1	93.8 × 43.3 × 19.6	1.25 (overlap of signals, H–C-5)
		C-10 × C-4 × C-6	
C-6	19.6	53.1 (C-5)	1.36 (m, H_2–C-6)
C-7	20.1	39.1 (C-8)	1.40 (m, H–C-7)
			1.48 (m, H–C-7)
C-8	39.1	20.1 × 37.3 (C-7 × C-9)	1.40 (m, H–C-8)
C-9	37.3	93.8 × 39.1 × 29.8 × 22.0	
		C-10 × C-8 × C-11 × C-25	
C-10	93.8	53.1 × 37.3 × 32.0	
		C-5 × C-9 × C-1	
C-11	29.8	32.3 × 37.3	2.23 (td, J = 14 Hz, 5.3, H–C-11)
		C-12 × C-9	1.12 (m, H–C-11)
C-12	32.3	29.8 × 36.5	1.54 (overlap of signals, H–C-12)
		C-11 × C-13	0.88 (overlapped by the methyl signal, H–C-12)
C-13	36.5	20.0 × 32.3 × 39.1 × 44.2	
		C-27 × C-12 × C-14 × C-18	
C-14	39.1	15.4 × 29.4 × 36.5	
		C-26 × C-15 × C-13	
C-15	29.4	39.1 × 34.6 (C-14 × C-16)	1.12 (m, H_2–C-15)
C-16	34.6	29.4 × 31.8	1.25 (overlap of signals, H_{ax}–C-16)
		C-15 × C-17	1.54 (overlap of signals, H_{eq}–C-16)
C-17	31.8	34.6 × 43.0 × 44.2 × 32.8	
		C-16 × C-19 × C-18 × C-28	
C-18	44.2	31.8 × 36.5	1.27 (bd, J = 14.2 Hz, H–C-18)
		C-17 × C-13	1.20 (bd, J = 14.2 Hz, H–C-18)
C-19	43.0	23.0 × 31.8	1.70 (m, H–C-19)
		C-20 × C-17	1.12 (m, H–C-19)
C-20	23.0	43.0 × 125.3	1.80 (m, H–C-20)
		C-19 × C-21	2.00 (m, H–C-20)
C-21	125.3	23.0 (C-20)	5.09 (t, J = 7.0 Hz, 1, H–C-21)
C-22	130.8	17.5 (C-29)	
C-23	22.8	43.3 (C-4)	0.88 (s, H_3–C-23)
C-24	24.2	43.3 (C-4)	1.00 (s, H_3–C-24)
C-25	22.0	37.3 (C-9)	1.23 (s, H_3–C-25)
C-26	15.4	39.1 (C-14)	1.02 (s, H_3–C-26)
C-27	20.0	36.5 (C-13)	1.04 (s, H_3–C-27)
C-28	32.8	31.8 (C-17)	0.89 (s, H_3–C-28)
C-29	17.5	130.8 (C-22)	1.59 (bs, H_3–C-29)
C-30	25.7		1.67 (bs, H_3–C-30)

of the roots of *Baccharis myriocephalla*[1] and *B. caprariaefolia*[1] in approximately 0.40% yield. The diethyl ether extract of the roots of *Baccharis elaegnoides*[1] furnished baccharis oxide in 2.8% yield.

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