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Note

Assignment of ^{13}C NMR data of methyl (+)-hardwickiate and its derivatives

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ABSTRACT: Despite the large number of clerodanes isolated as natural products in the last decade, the correct ^{13}C NMR chemical shift assignments of some carbons are still in doubt. In order to provide unambiguous assignments of the chemical shifts of clerodane diterpenes, a complete ^{13}C NMR spectral analysis of methyl (+)-hardwickiate and 14 hemisynthetic derivatives is reported. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{13}C NMR; methyl (+)-hardwickiate; clerodane derivatives

INTRODUCTION

In the course of our research on the isolation and chemical transformations of some readily available resinic acids from commercial copaiba oil,[1] one of the major components, (+)-hardwickiic acid, was isolated as the methyl ester (**1**) after treatment with diazomethane. Although the ^{13}C NMR data for the (−)-enantiomer have been reported,[2,3] we decided to perform an unambiguous assignment.

The assignment of the ^{13}C NMR resonance signals of **1** was based on general chemical shift arguments,[4,5] analogy with decalin models[6] and comparison with related clerodanes.[2,3,7–10] Surprisingly, in spite of the NMR data available for a large number of new natural clerodane diterpenes which have been isolated, we frequently found equivocated chemical shift assignments, mainly for carbons C-1, C-2, C-6, C-7, C-11 and C-12. We therefore decided to undertake a detailed study of the assignment of ^{13}C NMR data for **1**–**15** (Fig. 1), utilizing 1D (^{1}H, ^{13}C, ^{13}C DEPT) experiments and 2D (one-bond and long-range HETCOR and ^{1}H–^{1}H COSY) experiments on representative compounds of each series.

EXPERIMENTAL

^{1}H, ^{13}C and DEPT spectra were obtained for all compounds on a Bruker AC 300 spectrometer operating at 300 MHz for ^{1}H in 5 mm tubes at ambient temperature using $CDCl_3$ as the solvent and tetramethylsilane as an internal reference. ^{1}H spectra were obtained with the following conditions: 30° pulse, 2.26 s acquisition time, 1 s relaxation delay and 32K data points. A 45° pulse, 0.82 s acquisition time, 2 s relaxation delay and 64K data points were used to obtain the ^{13}C spectra. For the DEPT sequence, the $(2J)^{-1}$ delay was set at 3.45 ms and a relaxation delay of 2 s was used.

The homonuclear ^{1}H–^{1}H COSY spectra of **1**, **5** and **10** were obtain on a Bruker AC 300 spectrometer using the COSY-45 pulse sequence with 1024 data points in t_2, 256 increments in t_1 and a 2 s relaxation delay. The data were processed by sinusoidal multiplication in each dimension followed by symmetrization.

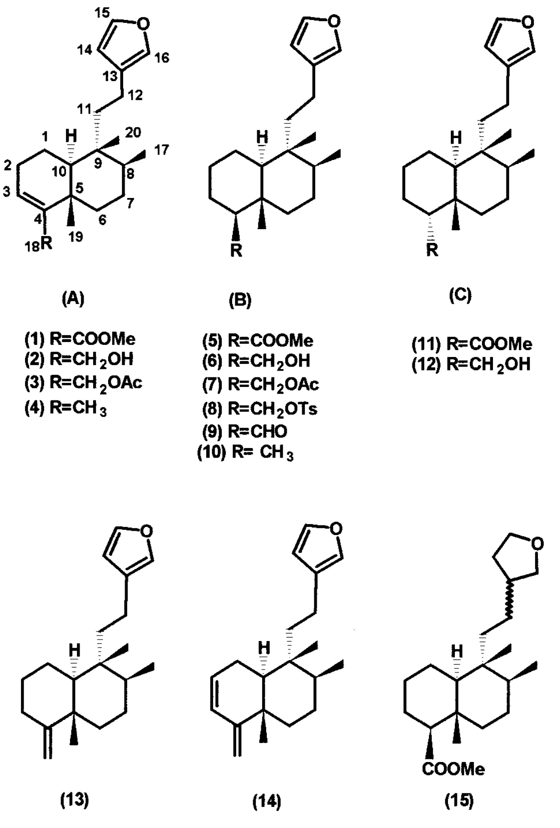

Figure 1. Structures of the compounds investigated.

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Table 1. ^{13}C chemical shifts of methyl (+)-hardwickiate (**1**) and its derivatives (**2–15**) (δ in ppm from TMS, $CDCl_3$ solutions)

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	17.5	18.2	18.2	18.2	21.0	21.5	21.4	21.3	21.1	21.5	21.0	21.5	21.8	23.6	21.5
2	27.2	26.6	26.7	26.7	26.2	26.9	26.7	26.5	26.0	27.4	22.6	22.4	28.7	128.3	26.5
3	137.1	122.3	126.2	120.6	24.9	25.4	25.7	25.3	21.9	30.8	24.3	23.6	33.1	128.8	25.0
4	142.7	148.2	142.9	143.7	57.6	54.4	50.5	50.6	64.0	46.1	53.9	51.3	160.6	157.1	57.6
5	37.7	37.5	37.9	37.9	37.3	37.0	36.9	36.9	38.1	37.2	36.7	36.9	40.1	37.7	37.4
6	36.0	36.4	36.2	36.6	39.9	39.5	39.3	39.2	39.9	39.3	38.3	37.0	37.4	37.2	39.9
7	27.3	27.3	27.2	27.4	27.1	27.3	27.3	27.1	26.9	27.4	27.2	27.3	27.4	27.3	27.2
8	36.3	36.3	36.3	36.2	36.6	36.5	36.5	36.3	36.6	36.6	36.5	37.0	36.7	36.7	36.6
9	38.9	38.8	38.8	38.4	38.9	38.9	38.8	38.8	39.0	38.5	38.6	38.8	38.8	38.9	38.8
10	46.6	46.4	46.3	46.1	49.3	49.7	49.6	49.6	49.4	49.8	39.9	42.2	48.7	43.5	49.3
11	38.7	38.6	38.6	38.5	38.3	38.5	38.5	38.4	38.5	38.8	38.3	38.2	38.4	38.2	37.1
12	18.2	18.2	18.2	18.2	18.1	18.1	18.1	18.0	18.1	18.1	18.2	18.2	18.1	18.0	26.2
13	125.8	125.9	125.9	125.2	125.6	125.9	125.9	125.8	125.8	125.9	125.9	125.7	125.7	125.6	40.1
14	111.2	111.2	111.2	110.7	111.0	111.2	111.2	111.2	111.2	111.1	111.1	111.0	111.0	111.0	32.8
15	142.9	142.9	142.9	142.3	142.7	142.9	142.9	142.9	143.0	142.6	142.5	142.7	142.6	142.6	68.1
16	138.6	138.6	138.6	138.0	138.4	138.6	138.6	138.6	138.7	138.4	138.5	138.4	138.4	138.3	73.8
17	16.0	16.0	16.0	16.1	16.0	16.1	16.1	16.0	16.0	16.2	16.0	16.0	16.6	15.9	16.0
18	168.0	63.1	65.1	17.9	175.0	63.6	65.8	72.1	206.7	13.2	175.8	62.1	102.5	107.0	175.3
19	20.7	21.4	21.3	19.8	14.8	15.2	15.0	15.0	16.3	15.2	21.6	23.0	20.9	22.0	14.8
20	18.3	18.3	18.1	18.2	18.1	18.1	18.2	18.1	18.1	18.2	18.1	18.2	18.1	18.1	18.2
CH_3/CO	50.5		171.3		50.9		171.7	144.8^{i}			50.8				51.0
Ac			21.2				21.1	130.0^{o}							
m-Ar								128.1^{m}							
p-Ar								133.5^{p}							
Ar-Me								21.7							

One-bond and long-range ^{13}C–^{1}H correlation experiments (HETCOR) of **1** and **5** were obtained on a Varian Unity spectrometer operating at 125.7 MHz. The spectra were acquired with proton decoupling and 128 increments in t_1 with coupling constants of 140 and 10 Hz for the one-bond and long-range correlations, respectively. The t_1 dimension was extrapolated to 512 using linear prediction and zero-filled to 2048 data points. Sinusoidal multiplication was used to process the 2048 × 4096 data points. One-bond and long-range ^{13}C–^{1}H HETCOR spectra of **11** were obtained on a Varian Gemini 2000 spectrometer operating at 75.4 MHz with 256 increments in t_1 and a relaxation delay of 1 s, and the 512 × 2048 data points were processed using exponential multiplication with LB = 3 Hz.

RESULTS AND DISCUSSION

All compounds were characterized by $[\alpha]_D^{25}$, MS, IR and ^{1}H NMR measurements and elemental analysis. Methly (+)-hardwickiate (**1**) was isolated from commercial copaiba oil[1] and the other compounds were prepared from **1** by reduction, hydrogenation, acetylation, oxidation, etc. The experimental procedures used will be published elsewhere.

The comparison of the ^{13}C NMR data for all the compounds reveals a certain constancy of the chemical shifts for the carbons of the B ring (C-6–C-9) and for side-chain carbons, except for **15**, which has a tetrahydrofuranyl group instead of a furan. As can be seen in Fig. 1, there are two major series with and without the Δ^3-decalin system for the AB ring (**1**–**4**). As mentioned before, the main discrepancies which we observed in the literature concern the possible exchange of the assignments for carbons C-2/C-7 and C-6/C-11. To confirm the assignments of these carbons, COSY (^{1}H–^{1}H), DEPT and one-bond and long-range HETCOR experiments were performed for **1**. From the ^{1}H–^{1}H COSY experiment, we could easily recognize the signals of H-2 at δ 2.18 and at 2.30 and of H7 at δ 1.43. From the correlations of these hydrogens in the HETCOR spectrum, we could assign the peaks at δ 27.2 and 27.3 to carbons C-2 and C-7, respectively. The ^{1}H signals of H-6 at δ 1.25 and 2.25 and of H-11 at δ 1.55 were correlated with the ^{13}C signals at δ 36.0 and 38.7, which were assigned to C-6 and C-11, respectively, in the long-range HETCOR spectrum.

From the long-range HETCOR experiment, a differentiation of the signal of quaternary carbons C-5 and C-9 was made. Through this experiment, the chemical shift at δ 37.7 was easily assigned to C-5 because of the correlations of this peak with the hydrogens of the C-19 methyl group at δ 1.28, H-6 (δ 1.15 and 2.29) and H-10 (δ 1.39). The observed correlations of the chemical shift at δ 38.9 with the hydrogens of the C-20 methyl group at δ 0.78, H-8 (δ 1.58) and H-10 (δ 1.39) confirmed the assigned of C-9. The assignments of the chemical shifts of C-6 and C-11 were also confirmed in the same way since the signals at δ 36.0 (C-6) and 38.7 (C-11) were correlated with the hydrogens of the methyl groups at C-19 (δ 1.28) and C-20 (δ 0.78), respectively.

The reduction of the double bond at C-3 to form **5**–**10** introduces a considerable modification of the A ring conformation, which causes predictable shift changes for C-3 and C-4 and also for the homoallylic carbons C-1, C-10 and C-6. The elimination of the endocyclic homoallylic effect causes a deshielding ($\Delta\delta \approx 3$) of C-1 and C-10; a deshielding of the same magnitude was also observed for C-6. Almost no effect was observed for the allylic carbons (C-2 and C-5). The long-range HETCOR experiment for **5** was particularly useful in this case, to confirm the assignment of the chemical shift of C-6 (which is deshielded by $\Delta\delta = 3.9$ ppm when compared with **1**), which resonates at δ 39.9 and is correlated with the hydrogens of the C-19 methly group at δ 0.95. The assignment of C-10 was also confirmed through the correlation of the chemical shift at δ 49.3 with the hydrogens of the C-19 and C-20 methyl groups at δ 0.95 and 0.63, respectively. The chemical shifts of these two methyl group were also correlated with the carbon signals at δ 37.3 and 38.9, respectively, confirming the previous assignments for the quaternary carbons C-5 and C-9. On comparison of the chemical shifts of the decalin system of **5** and **15**, we observed that they are very similar except for the side chain, as expected.

On comparison the ^{13}C chemical shifts of **11** and **12** with **5** and **6**, we can clearly observe the differences for C-2, C-10 and C-19 due to the change in orientation of the R group ($COOCH_3$ and CH_2OH) at C-4, from axial to equatorial. For **11** and **12**, we can observe a shielding of C-2 ($\Delta\delta \approx -4$) and C-10 ($\Delta\delta \approx -7$ to -9) due to the effect of the *γ-gauche* interaction with the R group and a deshielding of the C-19 methyl group ($\Delta\delta \approx 6$). We can also observe a slight shielding effect on C-4 ($\Delta\delta \approx -3$).

For **13** with $\Delta^{4(18)}$, we observed a significant modification of the ^{13}C chemical shifts for the carbons of the A ring when compared with the corresponding carbons of **10**. These chemical shifts are also very similar to those published for the related clerodane diterpene.[8] For **14**, the double bonds at $\Delta^{2,4(18)}$ causes a significant modification of the chemical shifts for the A ring carbons (see Table 1).

Our analysis suggests that the ^{13}C NMR data in Table 1 are correctly assigned and we hope that these compounds will serve as models for the assignment and characterization of similar compounds in further research.

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