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SHORT COMMUNICATION

Assignment of Carbon-13 and Proton Nuclear Magnetic Resonance Spectra of Madeirane Triterpenes

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The assignments of ^{13}C and ^{1}H NMR spectra for madeirane triterpenes are reported. The assignments were made on the basis of two-dimensional homonuclear ^{1}H, ^{1}H (COSY) and of ^{1}H detected ^{1}H, ^{13}C heteronuclear correlation (HMQC) and also from a series of one-dimensional selective INEPT experiments. © **1997 by John Wiley & Sons, Ltd.**

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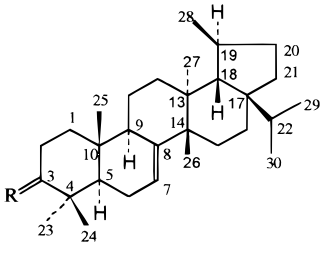
INTRODUCTION

We have been proceeding with the phytochemical study of *Euphorbia mellifera* Ait.[1–3] Among the compounds isolated, we have found a new type of pentacyclic triterpene skeleton named madeirane as represented by D:C-*friedo*-madeir-7-en-3β-ol (isomadeiranol, **1**) D:C-*friedo*-madeir-7-en-3-one (isomadeiranone, **3**), D-*friedo*-madeir-14-en-3β-ol (madeiranol, **4**), D-*friedo*-madeir-14-en-3-one (madeiranone, **6**) and compounds **2** and **5** obtained by acetylation of **1** and **4**, respectively.

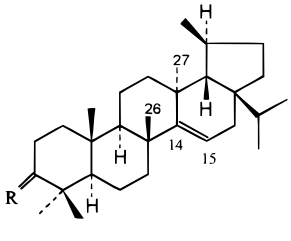
Their structures were determined by NMR, MS, IR and x-ray analysis. The madeirane skeleton differs from the derivatives of lupane or hopane by an unusual arrangement of the substituents in the cyclopentane ring.



1 R = H (α), OH (β)

2 R = H (α), CH_3 COO (β)

3 R = O



4 R = H (α), OH (β)

5 R = H (α), CH_3 COO (β)

6 R = O

The total assignment of the ^{13}C NMR spectra of compounds **1**–**6** is reported in this paper, together with the complete assignment of the ^{1}H NMR spectra of compounds **1** and **4**, which had been partially assigned previously[2] based on literature data.

EXPERIMENTAL

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity-300 spectrometer. The spectra were recorded at 25 °C in $CDCl_3$ as solvent, the concentration of all solutions being 20 mg ml^{-1}. The ^{1}H and ^{13}C chemical shifts are referenced to TMS, used as an internal standard.

Soft pulses in selective INEPT were generated by a DANTE sequence and the delays were optimized for long-range J_{CH} couplings of 4 and 8 Hz. The NOE 1D difference spectra were acquired with a saturation delay of 5 s and 1024 transients. The pure absorption one-bond proton–carbon correlation spectrum was obtained with the HMQC sequence with 256 × 2K complex points, SW = 6000 Hz, an acquisition time 0.2 s and 32 scans per increment with a relaxation delay of 2 s. The HMQC spectra were processed with a cosine squared filter in F_2 and F_1. The delay in the BIRD sequence was optimized for an average J_{CH} coupling of 135 Hz.

The conventional COSY experiment was run on a Varian Unity 500 MHz spectrometer as 512 × 2K complex points, SW = 3000 Hz, acquisition time 0.6 s, 8

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scans per t_1 increment, a relaxation delay of 1.5 s and a sine filter in both dimensions.

RESULTS AND DISCUSSION

For the assignment of the carbon skeleton of compounds **1** and **4**, we carried out HMQC experiments (^{1}H detected H, C correlation) and a series of selective INEPT experiments. In addition, the COSY spectra at 500 MHz were of great help for completing the carbon spectral assignments (Table 1). The chemical shifts of the attached protons are given in Table 2.

The olefinic carbons C-7 (δ 116.94) and C-8 (δ 145.38) and the hydroxylic carbon C-3 (δ 79.27) of **1** are readily identified from their chemical shifts. The methine proton H-3α (δ 3.24) shows COSY cross peaks with the two methylenic protons at carbon C-2 (δ 27.70), which are further correlated with the protons at carbon C-1 (δ 37.00).

In ring B of **1**, coupling of the methine proton H-7 (δ 5.45) with the two allylic protons at carbon C-6 (δ 24.15) and of these with the methine proton at C-5 (δ 50.26) made the assignment of both carbon resonances possible.

The identification of carbons C-9 (δ 48.54) and C-11 (δ 16.83) in ring C followed that of the methine H-9 proton (δ 2.12), which was deshielded by the nearby Δ^7 double bond.

In the five-membered ring, the entire proton system was assigned through the coupling of the methine proton H-19 (δ 2.10) with H-18 (δ 1.58) and H-20 (δ 1.26, 1.81). By analysis of the HMQC spectrum, the carbons resonances C-18 (δ 56.61), C-19 (δ 35.14), C-20 (δ 31.05) and C-21 (δ 35.86) were then assigned.

The remaining carbon resonances of **1** were assigned from a series of two- and three-bond selective INEPT experiments. Thus, by the selective irradiation of the methine resonances H-3 and H-7, the quaternary carbons C-4 and C-14 could be identified. Similar irradiations of Me-10β (δ 0.74) and of Me-14β (δ 0.92) produce responses at the carbons C-1, C-5, C-9, C-10 and C-8, C-13, C-14 and C-15 (δ 28.44), respectively.

The INEPT spectrum of **1** obtained by irradiation of Me-13α (δ 0.95) is not selective as the isopropyl methyl doublet at δ 0.94 and the methyl 4α singlet (δ 0.97) were also excited. Among the resonances observed, those of the carbons C-13, C-14 and C-18 could be identified. In the same way, excitation of the methine resonance H-22 (δ 1.69) allowed the assignment of the resonance of the quaternary carbon C-17 (δ 46.78) and of the methylene carbon C-16 (δ 22.14).

The strategy for assigning the carbon spectrum of **4** is identical with that used for the isomer **1**. Identification of the olefinic carbons C-14 (δ 157.53) and C-15 (δ

Table 1. ^{13}C NMR chemical shift assignments of madeirane triterpenes 1–6 ($CDCl_3$)

Carbon	1	2	3	4	5	6
C-1	37.00	36.72	38.35	37.84	37.47	38.39
C-2	27.70	24.27	34.91	27.23	23.51	34.16
C-3	79.27	81.23	216.85	79.10	81.06	217.51
C-4	38.87	37.80	47.75	38.80	37.91	47.57
C-5	50.26	50.50	52.02	55.54	55.62	55.76
C-6	24.15	24.01	24.56	18.82	18.71	19.95
C-7	116.94	116.84	116.93	32.45	32.41	32.25
C-8	145.38	145.61	145.61	39.07	39.03	39.00
C-9	48.54	48.56	48.14	49.56	49.44	48.90
C-10	35.26	35.21	35.33	38.03	37.71	37.71
C-11	16.83	16.90	17.10	17.19	17.18	17.10
C-12	35.26	35.32	35.25	41.79	41.67	41.02
C-13	37.03	37.11	37.07	29.72	29.70	29.69
C-14	40.45	40.53	40.63	157.53	157.42	157.03
C-15	28.44	28.52	28.64	118.19	118.25	118.46
C-16	22.14	22.20	22.15	30.37	30.33	30.28
C-17	46.78	46.86	46.80	50.34	50.31	50.24
C-18	56.61	56.72	56.70	63.53	63.53	63.50
C-19	35.14	35.21	35.20	34.15	34.13	34.08
C-20	31.05	31.12	31.06	33.44	33.42	33.37
C-21	35.86	35.93	35.91	38.52	38.50	38.51
C-22	33.56	33.60	33.57	34.48	34.47	34.42
C-23	27.59	27.60	24.56	28.06	28.01	26.12
C-24	14.72	15.87	21.57	15.49	16.61	21.52
C-25	13.09	13.17	12.78	15.33	15.38	14.71
C-26	22.95	23.00	23.11	25.16	25.20	24.84
C-27	24.14	24.27	24.28	19.64	19.59	19.62
C-28	25.79	25.79	25.74	24.46	24.45	24.47
C-29	17.35	17.35	17.30	19.05	19.03	19.01
C-30	17.03	17.07	17.02	16.76	16.75	16.71
CH_3COO—	—	21.30	—	—	21.26	—
CH_3COO—	—	170.97	—	—	170.94	—

Table 2. ^{1}H NMR chemical shift assignments of compounds 1 and 4 ($CDCl_3$)

	δ (ppm), J (Hz)	
	1	4
H-1	1.12, 1.68	0.93, 1.62
H-2	1.60, 1.64	1.56, 1.58
H-3 α	3.24 dd (J = 4.2, 11.4)	3.20 dd (J = 5.0, 11.0)
Me-4 α	0.97 s	0.97 s
Me-4 β	0.86 s	0.80
H-5 α	1.30	0.74
H-6	1.97 dd (J = 10.6, 18.8), 2.15 d (J = 18.8)	1.60, 1.48
H-7	5.45 m, $W_{1/2}$ = 11.0)	1.56, 1.61
Me-8 β	—	1.02 s
H-9 α	2.12	1.27
Me-10 β	0.74 s	0.92 s
H-11	1.48, 1.59	1.42, 1.58
H-12	1.45, 1.63	1.96, 1.28
Me-13 α	0.95 s	0.94 s
Me-14 β	0.92 s	—
H-15	1.48, 1.52	5.41 dd (J = 2.4, 8.0)
H-16	1.26, 1.48	1.63, 2.10 dd (J = 8.0, 16.3)
H-18 β	1.58	1.16 d (J = 4.9)
H-19 α	2.10	1.96
Me-19 β	1.08 d (J = 7.3)	1.04 d (J = 6.8)
H-20	1.26, 1.81	1.77, 1.17
H-21	1.26, 1.60	1.85, 1.18
H-22	1.69	1.45
Me-22	0.83 d (J = 6.8); 0.94 d (J = 6.8)	0.72 d (J = 6.8); 0.77 d (J = 6.8)

118.19) and of the hydroxylic carbon C-3 (δ 79.10) is obvious from their characteristic chemical shifts. The assignment of carbons C-19 (δ 34.15) and C-16 (δ 30.37) is also straightforward from the ^{1}H–^{13}C HMQC correlation spectrum.

The COSY cross peaks of proton H-3 (δ 3.20) with its vicinal protons at carbon C-2 (δ 27.23) and of proton H-19 (δ 1.96) with its vicinal protons at carbons C-18 (δ 63.53) and C-20 (δ 33.44) made possible the identification of the whole proton system in rings A and E.

The other carbon resonances of **4** were assigned from a series of selective INEPT experiments. Irradiation of the methine resonance H-3 provides response at the quaternary carbon C-4 (δ 38.80) and at methyl carbons Me-4α (δ 28.06) and Me-4β (δ 15.49). In the same way, irradiation of the methine H-15 produces responses at the quaternary carbons at δ 50.34, 39.07 and 29.72. The carbon resonances C-17 (δ 50.34) and C-13 (δ 29.72) were identified by irradiation of the isopropyl methyl doublet at δ 0.72 and of the methine H-19 (δ 1.96). Thus, the quaternary carbon at δ 39.07 was assigned to C-8.

In ring B, carbon C-5 (δ 55.54) was identified by irradiation of Me-4β (δ 0.80), which also allowed the identification of the methylenic carbon C-6 (δ 18.82) through the coupling of the attached protons with methine H-5 (δ 0.74). Furthermore, when the methyl resonance at δ 1.02 was irradiated, responses were observed at the quaternary carbons C-8 and C-14 and at the carbons C-7 (δ 32.45) and C-9 (δ 49.56). Hence, this methyl must be Me-8β. Irradiation of Me-13 (δ 0.94) is not selective as the close methyl singlet at δ 0.92 (Me-10β) is also affected. Among the responses observed are those of carbons C-13, C-14 and C-18 and of the methylene carbon C-12 (δ 41.79). Correlation of the protons at C-12 with those at C-11 (δ 17.19) in the COSY spectrum of **4** allowed the assignment of this high-field methylene carbon.

Table 3. Changes in chemical shifts of compounds 2, 3, 5 and 6 (δ, ppm) due to the substitution on C-3 relative to the δ-values of 3-OH madeirane compounds

Carbon	2 $\Delta\delta$ (OH→OAC)	3 $\Delta\delta$ (OH→O)	5 $\Delta\delta$ (OH→OAC)	6 $\Delta\delta$ (OH→O)
C-1	−0.3	+1.3	−0.3	+0.5
C-2	−3.4	+7.2	−3.7	+6.9
C-3	+1.9	+137.5	+1.9	+138.4
C-4	−1.0	+8.9	−0.9	+8.7
C-5	+0.2	+1.7	+0.1	+0.2
C-10	0	0	−0.3	−0.3
C-23	0	−3.0	0	−1.9
C-24	+1.1	+6.8	+1.2	+6.0
C-25	0	−0.3	0	−0.6

The stereochemistry at C-4 on both compounds **1** and **4** was derived from the NOE enhancements observed from H-3α to H-5α and Me-4α.

The natural product ketones **3** and **6** and the monoacetate derivatives **2** and **5** gave essentially the same ^{13}C spectra as the isomers **1** and **4** except for the chemical shifts of the carbons in ring A, which are dependent on the functional group present at C-3. The changes observed (see Table 3) are similar to those reported for other triterpenes.[4–8] The ^{13}C data for these derivatives are also presented in Table 1.

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REFERENCES

1. M. J. U. Ferreira, A. M. Lobo, C. A. O'Mahoney, D. J. Williams and H. Wyler, *J. Chem. Soc., Perkin Trans. 1* **1**, 185 (1990).
2. M. J. U. Ferreira, A. M. Lobo, C. A. O'Mahoney, D. J. Williams and H. Wyler, *Helv. Chim. Acta* **74**, 1329 (1991).
3. M. J. U. Ferreira, A. M. Lobo and H. Wyler, *Fitoterapia* **64**, 377 (1993).
4. R. C. Carpenter, S. Sotheeswaran and M. U. Sultanbawa, *Org. Magn. Reson.* **14**, 462 (1980).
5. N. Sakurai, Y. Yaguchi and T. Inque, *Phytochemistry* **26**, 217 (1987).
6. Y. Schun, G. A. Cordell, P. J. Cox and R. A. Howie, *Phytochemistry* **25**, 753 (1986).
7. S. A. Knight, *Org. Magn. Reson.* **6**, 603 (1974).
8. S. Mclean, W. F. Reynolds, J. P. Yang, H. Jacobs and L. L. Jean-Pierre, *Magn. Reson. Chem.* **32**, 422 (1994).

