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An acetylated monoterpene and a sesquiterpene alcohol from *Psiadia anchusifolia*

Anne Gauvin[a,*], Jacques Susperregui[b], Patrick Barth[c], Rémy Louis[c], Gérard Déléris[b], Jacqueline Smadja[a]

[a]*Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, Faculté des Sciences et Technologies, Université de la Réunion, 15 Avenue René Cassin, BP 7151, 97715 St Denis Messag Cedex 9, La Réunion, France*
[b]*INSERM U577, Biomatériaux et Réparation Tissulaire, Groupe de Chimie Bio-Organique, Université Victor Segalen Bordeaux 2, 146 Rue Léo-Saignat, 33076 Bordeaux, France*
[c]*Laboratoire de Géochimie Bioorganique UMR 7509 et Groupe Structure et Spectroscopie, Université Louis Pasteur, Institut de Chimie, 1 Rue Blaise Pascal, BP 296R8, 67008 Strasbourg Cedex, France*

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Abstract

Two compounds identified as 7,7-dimethyl-2-methylenebicyclo[3.1.1]heptan-6-ol acetate and 6,6,8,9-tetramethyltricyclo[3.3.3.0]-undec-7-en-2-ol were isolated from the essential oil of the fresh leaves of *Psiadia anchusifolia*. Their structures were determined by extensive NMR studies (^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HSQC, HMBC) as well as by X-ray crystallographic analysis.

Keywords: *Psiadia anchusifolia*; Asteraceae; Essential oil; Acetylated monoterpene; Sesquiterpene alcohol; 7,7-Dimethyl-2-methylenebicyclo[3.1.1]-heptan-6-ol acetate; 6,6,8,9-Tetramethyltricyclo[3.3.3.0]undec-7-en-2-ol

1. Introduction

The genus *Psiadia* Jacq. (Asteraceae) comprising aromatic plants is widely distributed in tropical and subtropical regions (Scott, 1991; Bosser et al., 1993). Its popular use may be explained by the presence of biologically active constituents either in the essential oils (Dennis, 1973; Gurib-Fakim et al., 1995, 2000; Mekkawi et al. 1984; Mossa et al., 1983; Ramanoelina et al., 1994) or as metabolites (Abou-Zaid et al., 1991; Al-Yahya et al., 1987; Canonica et al. 1967, 1969a,b; El-Domiaty et al., 1993; El-Feraly et al., 1990; Fortin et al., 2001; Jakobsen et al., 2001; Juma et al., 2001; Midiwo et al., 1997; Mossa et al., 1992; Robin et al., 1998, 2001; Wang et al., 1989a,b, 1992).

As part of our continuing phytochemical investigation of plants indigenous to Reunion Island, the chemical composition of the essential oil obtained from the leaves of *Psiadia anchusifolia* has been examined. No chemical or biological studies of this species have been reported and interest in its constituents was stimulated by the pharmacological action of related species. This study has led to the isolation of two new compounds, major constituents of the essential oil: an acetylated monoterpene (**1**) and a sesquiterpene alcohol (**3**) with an unusual [3.3.3]-propellane carbon skeleton. The present paper describes the isolation and structure elucidation of these two compounds by means of 1D and 2D NMR spectroscopic techniques including DEPT, HSQC and HMBC as well as by X-ray crystallographic analysis.

2. Results and discussion

GC–MS analysis of the essential oil of *Psiadia anchusifolia* revealed the presence of at least 64 volatile components. Compounds **1** and **3** (respectively 12.0 and 20.5% of the essential oil) whose complete structures could not be deduced from mass spectrometric data, were isolated by repeated column chromatography and identified as 7,7-dimethyl-2-methylenebicyclo[3.1.1]-

* Corresponding author. Tel.: +41-26300-8849; fax: +41-26300-9698.
E-mail address: mirka.macel@unifr.ch (A. Gauvin).

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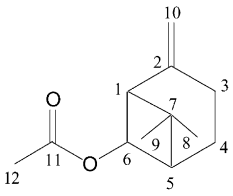

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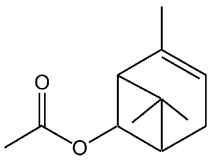

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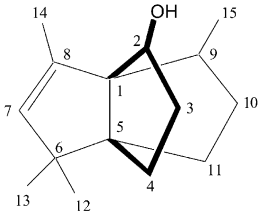

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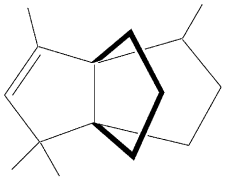

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heptan-6-ol acetate (**1**) and 6,6,8,9-tetramethyltricyclo[3.3.3.0]undec-7-en-2-ol (**3**).

Compound **1** was obtained as a colourless oil and its molecular formula was deduced as $C_{12}H_{18}O_2$ from EI-MS, indicating four degrees of unsaturation. IR spectral data revealed the presence of two multiple bonds: a carbonyl group at (1730 cm^{-1}) and a carbon–carbon double bond at (1645 and 900 cm^{-1}); the molecule was thus bicyclic. Its mass spectrum showed a peak at *m*/*z* 134 indicating the loss of acetic acid from the molecular ion (*m*/*z* 194). The presence of an acetate group in **1** was also suggested by the carbon resonance at *δ* 170.5 (*s*) and 21.5 (*q*), and it was confirmed by the HMQC correlation of the latter resonance to the three proton resonance at δ 1.99 (*s*). The monoterpene structure of compound **1** was directly inferred from its ^{13}C NMR spectrum with the aid of DEPT experiment (Table 1) where it was possible to account for all ten carbons after subtraction of the two carbons ascribed to an acetate group; two methyl groups (*δ* 27.5, 23.3), three methylenes (*δ* 107.8, 23.7, 23.5), three methines (*δ* 77.6, 56.1, 45.2) and two non-protonated carbon atoms (*δ* 149.5, 40.0). The comparison of the ^1H and ^{13}C NMR data with those of other acetylated monoterpenes reported in the literature indicated that **1** had a structure similar to that of *trans*-chrysanthenyl acetate **2** (Neszmelyi et al., 1992), but different in the position of the carbon–carbon double bond. The olefinic carbon signal at *δ* 149.5 (*s*) and 107.8 (*t*) suggested presence of an *exo*methylene double bond, which was confirmed by two signals in the ^1H NMR spectrum at *δ* 4.61 (1H, *s*) and 4.68 (1H, *s*). The location of the carbon–carbon double bond was deduced from the important correlations observed between the olefinic protons at C-10 (*δ* 4.61, 4.68) and the carbon C-1 (*δ* 56.1) and between the proton at C-6 (*δ* 4.65) and the carbon C-2 (*δ* 149.5). Thus the structure of **1** was established to be 7,7-dimethyl-2-methylenebicyclo[3.1.1]heptan-6-ol acetate.

Compound **3** was isolated as colourless to white needles. Its spectral data indicated that its structure is close to those of modhephene (**4**) and its derivatives. These compounds with an unusual [3.3.3]-propellane carbon skeleton were isolated from only a few genera such as *Berkheya* (Bohlmann et al., 1979), *Liabum* (Bohlmann et al., 1980), *Pluchea* (Reyes-Trejo and Joseph-Nathan, 1999), *Pulicaria* (San Feliciano et al., 1988) and *Silphium* (Bohlmann and Jakupovic, 1980). All these genera, like the genus *Psiadia*, belong to the family Asteraceae.

The EI-MS spectrum of **3** showed a molecular ion peak at *m*/*z* 220 compatible with the molecular formula $C_{15}H_{24}O$ and indicating four degrees of unsaturation. The ^1H NMR of **3** (Table 2) showed the presence of two

Table 1
^1H and ^{13}C NMR data for compound **1**

Position	DEPT	δ_C	δ_H (*J* in Hz)	HMBC H to C
1	CH	56.1	2.60 *s* (6.3)	C-2, C-5, C-6, C-8, C-9, C-10
2	C	149.5		
3	CH_2	23.7	1.91 *m*, 2.01 *m*	C-4
4	CH_2	23.5	2.26 *m*, 2,47 *m*	C-3, C-6
5	CH	45.2	2.11 *m*	C-1, C-4, C-6
6	CH	77.6	4.65 *s*	C-2, C-4, C-7, C-11
7	C	40		
8	CH_3	23.3	0.76 *s*	C-1, C-5, C-7, C-9
9	CH_3	27.5	1.39 *s*	C-1, C-5, C-7, C-8
10	CH_2	107.8	4.61 *s*, 4.68 *s*	C-1
11	C	170.5		
12	CH_3	21.5	2.04 *s*	C-11

Table 2
^1H and ^{13}C NMR data for compound **3**

Position	DEPT	δ_C	δ_H (*J* in Hz)	HMBC H to C
1	C	76.4		C-3, C-4, C-7, C-9, C-10[a], C-11[a], C-14, C-15
2	CH	78.8	4.23 *t* (3.2)	C-3, C-4, C-5, C-8
3	CH_2	34.5	(1.50, 1.80)[b]	
4	CH_2	34.7	(1.30, 2.05)[b]	
5	C	66.2		C-2, C-3, C-4, C-7, C-10[a], C-11[a], C-12, C-13
6	C	46.2		C-7, C-12, C-13
7	CH	136.4	4.81 *s*	C-1, C-5, C-6, C-8, C-12, C-13, C-14
8	C	140.2		
9	CH	44.9	1.8	C-10[a], C-11[a], C-15
10	CH_2	37.3[c]	(1.54, 1.75)[b,c]	
11	CH_2	37.6[c]	1.75[b,c]	
12	CH_3	29.3	0.95 *s*	C-5, C-6, C-7, C-13
13	CH_3	26	0.96 *s*	C-5, C-6, C-7, C-12
14	CH_3	14.1	1.67 *d* (1.5)	C-1, C-6, C-7, C-8, C-12, C-13
15	CH_3	16	1.30 *d* (6.7)	C-1, C-9, C-10[a], C-11[a]

[a] Ambiguous couplings due to the overlapping of C-10 and C-11 signals.
[b] Approximate central values due to overlapped signals.
[c] Assignments marked with the same superscript are interchangeable.

tertiary methyl groups [δ 0.95, 0.96 (each 3H, *s*)], a secondary methyl group [δ 1.30 (3H, *d*, $J=6.7$ Hz)], a vinyl methyl group [δ 1.67 (3H, *d*, $J=1.5$ Hz)], a methine proton adjacent to an oxygen atom [δ 4.23 (1H, *t*, $J=3.2$ Hz)] and an olefinic proton [δ 4.81 (1H, *s*)]. The ^{13}C NMR spectra, including DEPT (Table 2), exhibited fifteen signals due to four methyls (δ 14.1, 16.0, 26.0, 29.3), four methylenes (δ 34.5, 34.7, 37.3, 37.6), two methines including an oxygen-bearing carbon (δ 44.9, 78.8), three quaternary carbons (δ 76.4, 66.2, 46.2) and a trisubstituted double bond (δ 136.4, 140.2). The degree of unsaturation in combination with the presence of only one multiple bond, a carbon–carbon double bond, suggested a tricyclic sesquiterpenoid. The proton and carbon connectivities deduced from HSQC and HMBC experiments (Table 2) led to the modhephene (**4**) framework (Bohlmann et al., 1980). A comparison of the ^{13}C NMR spectra of **3** and **4**, revealed that the carbon signals were almost identical. However, one of the ^{13}C methylene resonances of **4** was absent and was replaced by the resonance of a hydroxyl bearing carbon as also shown by an IR absorption band at 3500 cm^{-1}. The location of the hydroxyl group at C-2 was determined from the following long-range HMBC couplings: the methine proton at C-2 (δ 4.23) with the carbons C-3 (δ 34.5), C-4 (δ 34.7), C-5 (δ 66.2) and C-8 (δ 140.2). Based on the above discussion **3** was identified as 6,6,8,9-tetramethyltricyclo[3.3.3.0]undec-7-en-2-ol.

This structure was confirmed by X-ray crystallographic analysis. In the crystalline state, the four molecules $C_{15}H_{24}O$ of the asymmetric unit are linked together through four hydrogen bonds (Fig. 1) between the four hydroxyl groups (distance O↔O 2.7 Å). These four molecules all have the same conformation. A perspective view of the solid-state conformation is shown in Fig. 2. This structure establishes unambiguously the relative configuration of 6,6,8,9-tetramethyltricyclo-[3.3.3.0]undec-7-en-2-ol for **3**. The molecule has an unusual tricyclic [3.3.3]-propellane carbon skeleton containing one cabon–carbon double bond, as in modhephene. Two five-membered rings have the same envelope conformation and thus the flaps point out in the same direction. The bond distances are 1.598(4) Å (central C-C) and 1.319(5) Å (carbon–carbon double bond). This is the first structure of a molecule belonging to the tricyclo[3.3.3.0]undecane family determined by X-ray crystallography.

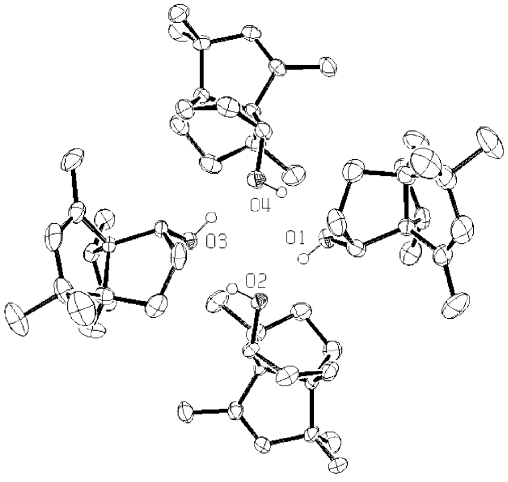

Fig. 1. ORTEP drawing for the asymmetric unit linked together through four hydrogen bonds. Displacement ellipsoids are shown at the 50% probability level. The CH_2 and CH_3 hydrogen atoms are omitted for clarity.

3. Experimental

3.1. General

Melting points are uncorrected and were determined on a Kofler apparatus. Specific rotations were measured at 20 °C using a Perkin-Elmer polarimeter 141. IR spectra were recorded on a Bruker IFS25 spectrometer. The ^{1}H (500.12 MHz) and ^{13}C (125.77 MHz) NMR spectra were recorded in $CDCl_3$ on a Bruker Avance spectrometer and the chemical shifts are reported in ppm relative to TMS as internal standard. The number

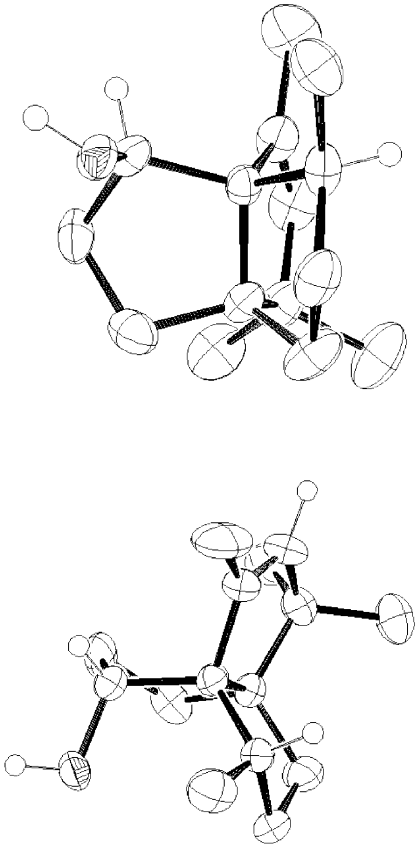

Fig. 2. X-ray structure of **3**. Displacement ellipsoids are shown at the 50% probability level. The CH_2 and CH_3 hydrogen atoms are omitted for clarity.

of attached protons for ^{13}C signals was determined using the DEPT pulse sequence. Inverse detected heteronuclear correlations were measured using the HSQC (optimized for $^{1}J_{CH} = 145$ Hz) and HMBC pulse sequences with a pulse-field gradient. COSY-45 spectra were used to determine the proton-proton connectivities. GC–MS analyses were carried out using a Hewlett-Packard chromatograph type 6890 series equipped with a Supelcowax capillary column (60 m×0.20 mm i.d., film thickness: 0.20 μm) and coupled to a HP 6890 mass selective detector. The MS detector was used in the EI mode with an ionization voltage of 70 eV. The GC conditions are: oven temperature programme, 60 °C rising at 4 °C/min to 230 °C, held for 30 min; ion source temperature, 280 °C; injector temperature, 250 °C; split ratio, 1:20; carrier gas, helium; flow rate, 0.7 ml/min. CC was performed on silica gel 60 (0.040–0.063 mm, Merck).

3.2. Plant material

Leaves of *Psiadia anchusifolia* were collected in April 2000 in the volcano area of Reunion Island (altitude 2300 m). The plant was identified by Dr. Dominique Strasberg, UMR Peuplements Végétaux et Bio-Agresseurs en Milieu Tropical, University of Reunion Island. A voucher specimen (# PAN00) is deposited in the Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, University of Reunion Island (DOM - France).

3.3. Extraction and isolation

The fresh leaves of the plant were subjected to hydrodistillation for 4 h, in a Clevenger-type apparatus. The essential oil was taken up in CH_2Cl_2, dried over Na_2SO_4 and stored in a cool place at 4 °C. The yellowish oil was obtained in 0.09% yield. The essential oil (1 g) was first chromatographed on a silica gel flash column eluted successively with *n*-hexane (300 ml) and Et_2O (300 ml). The Et_2O fraction containing the constituents of interest, was evaporated under reduced pressure then submitted to repeated column chromatography over flash silica gel with *n*-hexane–Et_2O gradient to afford 27 mg of **1** (colourless oil) and 33 mg of **3** (colourless to white needles). The fractions were monitored by GC-MS.

3.4. 7,7-Dimethyl-2-methylenebicyclo[3.1.1]heptan-6-ol acetate (1)

Colourless oil: IR ν_{max}^{film} cm^{-1}: 1730 (C=O), 1645, 900; EI-MS 70 eV, *m*/*z* (rel. int.): 194 $[M]^+$ (2), 151 (17), 134 $[M–CH_3COOH]^+$ (27), 119 (8), 109 (65), 91 (20), 81 (16), 69 (18), 53 (10), 43 (100); NMR spectral data: see Table 1.

3.4.1. 6,6,8,9-Tetramethyltricyclo[3.3.3.0]undec-7-en-2-ol (3)

Colourless to white needles: mp 62 °C; $[\alpha]_D^{20}$ +8° (CH_2Cl_2; *c* 1); IR ν_{max}^{KBr} cm^{-1}: 3500 (OH); EI-MS 70 eV *m*/*z* (rel. int.): 220 $[M]^+$ (5), 205 $[M–CH_3]^+$ (32), 187 $[M–CH_3–H_2O]^+$ (25), 174 (7), 163 (100), 145 (21), 135 (22), 119 (60), 107 (32), 105 (32), 91 (41), 77 (24), 65 (11), 55 (20), 75 (33); NMR spectral data: see Table 2.

3.5. X-ray analysis of 3

A colourless crystal of 0.20×0.20×0.12 mm obtained by recrystallisation from CH_2Cl_2 (sealed tube) was mounted on a CAD 4 kappa CCD Nonius diffractometer at 173 K. Intensities measurements were carried out up to $\theta = 30.01$ using Φ scan mode. A total of 14,189 reflections was collected, of which 8770 with $I > 3\sigma(I)$ were used for structure determination and refinement (576 variables). The crystal data are: monoclinic, $a = 13.2952(2)$, $b = 14.2808(3)$, $c = 14.3791(3)$ Å, $\beta = 92.073(5)°$, $V = 2728.32$ (9) $Å^3$, space group $P12_11$ (International Tables no. 4), $Z = 2$, $d = 1.07$ $g.cm^{-3}$ with four molecules $C_{15}H_{24}O$ in the asymmetric unit. The crystal structure and relative configuration were solved using the MOLEN (1997) package. All hydrogen atoms were located satisfactorily. A final weighted anisotropic full-matrix refinement gave $R = 0.061$ and $R_w = 0.077$, largest peak in final difference 0.573 e $Å^{-3}$, goodness-of-fit $S = 1.329$. Crystallographic data, atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center (CCDC) (deposition number CCDC 232103). These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

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