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Highly oxygenated bisabolenes and an acetylene from *Matricaria aurea*

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Abstract

Reinvestigation of the aerial parts of *Matricaria aurea* led to the isolation of three new bisabolenes and a new acetylene. The structures of the four compounds, namely (1*R**,2*R**,3*R**,6*R**,7*R**)1,2,3,6,7-pentahydroxy-bisabol-10(11)-ene, (1*R**,2*R**,3*R**,6*R**,7*R**)1,2,3,6,7-pentahydroxy-1-acetoxy-bisabol-10(11)-ene, (1*R**,2*R**,3*R**,6*R**,7*R**)1,2,3,6,7-pentahydroxy-2-acetoxy-bisabol-10(11)-ene and (3*S**,4*S**,5*R**)-(*E*)-3,4-dihydroxy-2-(hexa-2,4-diynyliden)-1,6-dioxaspiro-(4,5)decane, were deduced from the high field NMR studies.

Keywords: *Matricaria aurea*; Asteraceae; Sesquiterpenes; Bisabolenes; Acetylene

1. Introduction

The interest in the genus *Matricaria* (Asteraceae, Tribe Anthemideae) has dramatically increased since its constituents have high therapeutic effects as anti-inflammatory, antiphlogistic and antimicrobial agents (Cekan, Herul, & Sorm, 1957; Yamazaki, Miyakado, & Mabry, 1982; Bettray, & Vomel, 1989; Carle, Fleischhauer, Beyer, & Reinhard, 1990; Gasic, & Luckic, 1990). The oil has been reported to have bactericidal and fungicidal activities, particularly against Gram-positive bacteria and *Candida albicans*. Chemical investigation of members of the genus *Matricaria* gave sesquiterpenes, flavonoids, coumarins and spiroethers (Craker, & Simon, 1986). Recently, the effects of essential oils of chamomile on histamine release from rats mast cells have been reported (Miller, Wittstock, Lindequist, & Teuscher, 1996). Previous work on the chemical constituents of *M. aurea* gave two new bisabolenes (Ahmed, Jakupovic, Abou El-Ellea, Seif El-Din, & Hussein, 1993).

2. Results and discussion

Reinvestigation of the air-dried aerial parts of *M. aurea* afforded three new bisabolenes, **1**, **3** and **4**, and a new acetylenic compound, **5**. The structure of **1** was determined on the basis of its spectral data, ^{1}H-, ^{13}C-NMR and DEPT and from comparison with the previously reported compound **2** (Ahmed et al., 1993). The main difference between **1** and **2** was found in the absence of the acetyl group, in the ^{1}H-NMR spectrum of **1**. This followed by change in the chemical shifts of some signals, for example H-15 appeared at δ 1.11 in **1**, instead of 1.54 in **2**. Moreover, H-1 and H-2 could be detected at δ 3.70 and 3.73, respectively. The other signals were close to those of **2**. In particular, the ^{13}C-NMR and DEPT experiments were conclusive in the assignment of the entire structure and displayed fifteen carbons. The resonance of C-1, C-2, C-3 and C-4 at δ 82.6, 80.7, 72.9 and 39.1, respectively, supported the absence of the acetyl group at C-3, in **1**. The observed coupling constants of H-1 and H-2, in **1**, established

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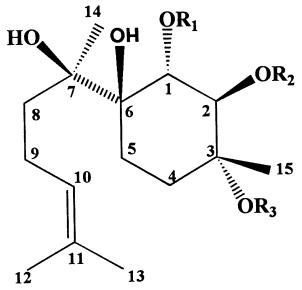

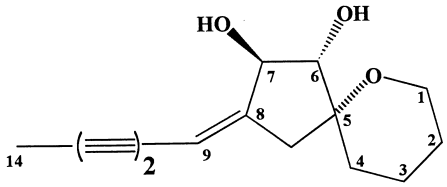

5

1 $R_1 = R_2 = R_3 = H$

2 $R_1 = R_2 = H$, $R_3 = Ac$

3 $R_1 = Ac$, $R_2 = R_3 = H$

4 $R_1 = R_3 = H$, $R_2 = Ac$

their stereochemistry as those of **2**, Table 1. Moreover, the molecular formula of **1** has been determined to be $C_{15}H_{28}O_5$ by EI mass spectrum, and exhibited a molecular ion peak at m/z 288 $[M]^+$ and two fragment peaks at m/z 270 $[M–H_2O]^+$ and 252 $[M–2H_2O]^+$.

The ^1H NMR spectra of **3** and **4** demonstrated the presence of an acetyl group at δ 2.17 and 2.08, respectively, similar to **2**. On the other hand, careful investigation of the ^1H-NMR spectra of both compounds showed some differences. The main difference was the chemical shifts of H-1 and H-2 which appeared at δ 4.42 and 3.60, respectively, in **3** and at δ 3.50 and 5.25, respectively, in **4**. These data suggested that the acetyl group must be at C-1 in **3** and C-2 in **4**. Furthermore, the chemical shifts of the carbon signals of C-1, C-2 and C-3 in the ^{13}C-NMR spectrum of **3**, compared to **2**, agreed with the proposed location of the acetyl group at C-1 (Table 2). The EI mass spectra of **3** and **4** were identical and gave an ion peak at m/z 330 $[M]^+$ and two fragments at m/z 312 $[M–H_2O]^+$ and 287 $[M–acetate]^+$.

Compound **5** displayed a different ^1H-NMR spectrum from those of **1**–**4**. Spin decoupling of **5** suggested that the compound has an acetylene skeleton, a common class of metabolite in the tribe Anthemideae (Gasic, & Luckic, 1990; Marco, Sanz,

Table 1
^1H-NMR of compounds **1**, **3** and **4** (400 MHz, $CDCl_3$, δ-value)[a]

Protons	1	3[b]	4
H-1	3.70 d (4)	4.42	3.50 br s
H-2	3.73 dd (4 and 2)	3.60	5.25
H-4	2.15–2.03 m	2.03	2.18
		1.58	2.05
H-5	2.02–2.15 m	2.11	2.05
	1.55 m	1.70	1.72
H-8	1.40 m	2.11	1.82
	1.20 m	1.70	1.33
H-9	2.15–2.02 m	2.10 m	2.05–2.00 m
H-10	5.14 br t (7)	5.13	5.33
H-12	1.68 br s	1.63	1.69
H-13	1.62 br s	1.61	1.62
H-14	1.32 s	1.29	1.23
H-15	1.11 s	1.14	1.24
OAc		2.17	2.08

[a] Coupling constant in parentheses (Hz).
[b] Assigned by ^1H–^1H COSY.

Table 2
^{13}C-NMR of compounds **1** and **3** (100.6 MHz, $CDCl_3$,δ-value)[a]

Carbons	1	3
C-1	82.6 d	87.2
C-2	80.7 d	79.3
C-3	72.9 s	72.2
C-4	39.1 t	39.1
C-5	28.3 t	28.3
C-6	76.8 s	76.5
C-7	72.2 s	71.9
C-8	27.4 t	27.9
C-9	22.3 t	21.1
C-10	125.0 d	124.9
C-11	131.5 s	131.5
C-12	25.7 q	25.7
C-13	17.6 q	17.6
C-14	22.7 q	22.4
C-15	22.9 q	23.0
OAc		172.6, 21.2

[a] Assigned by DEPT.

Jakupovic, & Huneck, 1990). The two characteristic signals of the terminal methyl and the olefinic proton were detected at δ 1.98 (H-14) and 4.73 (H-9), respectively. Moreover, irradiation of this olefinic signal changed the double doublets at δ 4.60 to a doublet ($J=7$ Hz, H-7). Irradiation of the signal at δ 4.60 changed a doublet at δ 3.94 (H-6) to a singlet. Irradiation of the oxygenated methylene group at δ 4.17 and 3.96, H-1, led to the assignment of the other protons (see Section 3). The stereochemistry of H-6 and H-7 could be easily deduced by comparison of their coupling constants with the diacetate, which has been isolated from *Chrysanthemum lavandulifolium* (Asteraceae, tribe Anthemideae) (Marco et al., 1990). The molecular formula of **5** was found to be $C_{14}H_{20}O_4$ from the EI mass spectrum, which exhibited a molecular ion peak at *m*/*z* 252.

The antimicrobial activity of the compounds **1**, **3** and **5** was evaluated against the test organisms, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* (Table 3). It is of interest to mention that the bioassay results is in agreement with that previously reported for oils of members of the genus *Matricaria*.

3. Experimental

3.1. Plant material

Matricaria aurea (Loefl.) Sch. Bip were collected from Bourg El-Arab, Alexandria, Egypt, in April 1995. Voucher specimens (A. Ahmed 101) are deposited at the Department of Botany, El-Minia University.

3.2. Extraction

The whole plant (1.6 kg) of *M. aurea* was extracted with CH_2Cl_2–MeOH (1:1) and evaporation of the solvent gave 10 g of a green gummy material. Separation of the products on silica gel column using CH_2Cl_2–hexane step gradient gave two main fractions. The first (100%, CH_2Cl_2) was purified by Sephadex LH-20 column to give 5 mg of **3** and 10 mg of **4**. The second (CH_2Cl_2–MeOH, 10:1) afforded a crude material which was separated into two compounds by TLC (CH_2Cl_2–MeOH, 15:1) and the isolated compounds were purified by HPLC (RP8, flow rate 3 ml/min, MeOH–H_2O, 55:45) to give 12 mg of **1** and 3 mg of **5**.

3.3. Physical and spectral data

3.3.1. (rel) (1R*,2R*,3R*,6R*,7R*)1,2,3,6,7-Pentahydroxy-bisabol-10(11)-ene (1)

IR$\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3580, 3550, 1020. EIMS *m*/*z*

Table 3
Inhibition zones in mm and minimum inhibitory concentration (MICs) mg/ml of compounds **1**, **3** and **5** and the reference antibiotics

Compound (concentration)	*S. aureus*	*E. coli*	*C. albicans*
	inhibition zones[a]		
1 (2 mg/ml)	22.5	24.0	28.0
1 (1 mg/ml)	12.0	19.0	21.0
3 (2 mg/ml)	20.5	26.0	28.0
3 (1 mg/ml)	12.0	20.0	23.0
5 (2 mg/ml)	13.0	19.0	23.0
5 (1 mg/ml)	12.0	15.0	20.0
Reference antibiotic (0.375 mg/ml)	27.0	34.0	–
Nystatin (0.1 mg/ml)	–	–	26.0
Diluent	12.0	19.0	14.0

[a] Average of two determinations.

(rel. int.): 288 $[M]^+$ (0.2), 270 $[M-H_2O]^+$ (5.4), 252 $[M-2H_2O]^+$ (8.6), 234 $[M-3H_2O]^+$ (11.0), 187 (58), 127 (100). $[\alpha]_D^{24}$: −39.9 ($c=0.36$, $CHCl_3$).

3.3.2. (rel) (1R,2R*,3R*,6R*,7R*)1,2,3,6,7-Pentahydroxy-1-acetoxy-bisabol-10(11)-ene (**3**)*

IR$\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3570, 1710, 960. EIMS *m/z* (rel. int.): 330 $[M]^+$ (21.0), 312 $[M-H_2O]^+$ (2.4), 287 [M–acetoxy]$^+$ (7.5), 68 (100). $[\alpha]_D^{24}$: −24.1 ($c=0.45$, $CHCl_3$).

3.3.3. (3S,4S*,5R*)-(E)-3,4-Dihydroxy-2-(hexa-2,4-diynyliden)-1,6-dioxaspiro-(4,5)decane (**5**)*

IR$\nu_{max}^{CHCl_3}$ cm^{-1}: 3510, 3500, 2210, 1645, 1220, 880. EIMS *m/z* (rel. int.): 252 $[M]^+$ (9.5), 234 $(M-H_2O)^+$ (21), 216 (M–2H_2O) (11) 127 (82), 111 (100). ^{1}H-NMR (400 MHz, $CDCl_3$, δ-value): 4.73 (dq, $J=2$ and 1 Hz, H-9), 4.60 (dd, $J=7$ and 2 Hz, H-7) 4.17 (ddd, $J=5$, 8, 8 Hz, H-1_a), 3.96 (ddd, $J=14$, 8, 8 Hz, H-1_b), 3.94 (d, $J=7$ Hz, H-6), 2.30–2.15 (m, H-3 and H-4), 1.99 (m, H-2), 1.98 (S, H-14). $[\alpha]_D^{24}$: −19.8 ($c=0.22$, EtOH).

3.4. Bioassay

The antimicrobial activity of compounds **1**, **3** and **5** was determined against *S. aureus* ATCC 29737, *E. coli* NCTC 10418 and *C. albicans* ATTCC 10231 using cup plate agar diffusion assay (United State Pharmacopoeia XXII, 1990). The media used were nutrient agar (Oxoid) for *S. aureus* and *E. coli* and Sabourand dextrose agar (Oxoid) for *C. albicans*. The overnight culture of each organism was diluted in saline to contain $\cong 10^6$ CFU/ml. An aliquot of 3 ml of the diluted culture was spread onto the surface of nutrient agar plate (40 ml each) and the excess bactrial suspension was withdrawn. The plates were incubated at 37°C for 30 min then cups were cut into the agar, each cup received 150 μl of either the corresponding isolated compounds or their diluted solutions. Then, the plates were incubated at 37°C for 24 h. The resultant zones of inhibition were measured and recorded in Table 3.

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