Two New Eudesmanolides from the Flowers of *Achillea millefolium*

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Two new eudesmanolides, 3β-acetoxy-1β,4α-dihydroxy-11αH-eudesman-12,6α-olide (**1**) and 3β-acetoxy-1β-hydroxy-11αH-eudesman-4(15)-en-12,6α-olide (**2**), were isolated from the flowers of *Achillea millefolium*, their structures were established on the basis of spectral analysis.

Introduction. – The genus *Achillea* (Compositae, Anthemideae) comprises over 100 species mainly distributed in the northern hemisphere, usually represented by small herbs. *Achillea millefolium* LINNAEAS (English name: yarrow), one of the most abundantly occurring species, has been important for a long time as a drug in traditional and modern medical practice [1][2]. The previous phytochemical investigation indicated that sesquiterpenoids and flavonoids were major components in the plant [3–7]. As part of our efforts screening bio-active agents with potential anti-tumor activities from *A. millefolium*, this report describes the isolation and structural elucidation of two new eudesmanolides **1** and **2** from the flowers of *A. millefolium*. Their structures were elucidated by extensive analysis of their spectral data (*Fig. 1*).

Results and Discussion. – Compound **1** was obtained as a gum. The HR-FAB-MS spectrum of **1** exhibited a *quasi*-molecular ion at *m*/*z* 365.1360 corresponding to the molecular formula $C_{17}H_{26}O_6$. The ^{1}H-NMR spectrum disclosed a total of 24 H-atoms attached to C-atoms, indicating the presence of two OH groups. Since the molecular formula contains five C=C equivalents, while only two C=O resonances and no olefinic

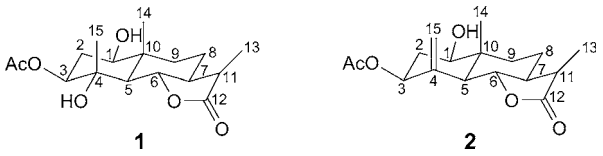

Fig. 1. *Structures of compounds* **1** *and* **2**

C-atom could be observed in the ^{13}C-NMR spectrum, the compound must be tricyclic. The ^{1}H-NMR spectral data (*Table 1*) showed two *singlet*s at δ(H) 1.00 (*s*) and 1.38 (*s*), and a *doublet* at δ(H) 1.20 (*d*) revealing the presence of three Me groups, while one *singlet* at δ(H) 2.09 (*s*) indicated the presence of an AcO group. The same spectrum showed a typical *doublet* of *doublet* for H–C(6) at δ(H) 4.38 (*dd*). This H-atom correlated with the C-atom at δ(C) 78.8 in the HSQC spectrum, indicating a C(6)-lactonized sesquiterpene lactone. The ^{1}H- and ^{13}C-NMR data of **1** are similar to those described for matricolone, but some chemical shift differences at C(7), C(11), C(12), and C(13) in both ^{1}H- and ^{13}C-NMR spectra were observed. These differences indicated that this compound should be an isomer of matricolone at Me(13). The β-orientation of Me(13) was definitely confirmed by the NOESY correlations observed, H–C(6)/MeC(13,14,15), and H–C(7)/H–C(11), as well as an absent important NOESY correlation H–C(6)/H–C(11), which was observed in matricolone [8] (*Fig. 2*). This conclusion was further supported by the chemical shift of C(13) at δ(C) 9.7, which was consistent with the reported value in the literature, *ca.* δ(C) 9.5 for β-orientation and δ(C) 12.5 for α-orientation [9]. Full assignments of the H- and C-atom signals were secured by ^{1}H,^{1}H-COSY, HSQC, and HMBC spectral analysis. Thus, compound **1** was identified as 3β-acetoxy-1β,4α-dihydroxy-11αH-eudesman-12,6α-olide, as shown in *Fig. 1*.

Compound **2** was isolated as a gum. The HR-FAB-MS spectrum of **2** exhibited a *quasi*-molecular ion at *m*/*z* 347.1255 corresponding to the molecular formula $C_{17}H_{24}O_5$, which accounts for the six degrees of unsaturation in the molecule. The ^{1}H-NMR spectral data of **2** (*Table 2*) displayed one *singlet* at δ(H) 0.83 (*s*), and a *doublet* at δ(H) 1.20 (*d*) revealing the presence of two Me groups; one *singlet* at δ(H) 2.12 (*s*) indicated the presence of an AcO group, which was further confirmed by the signals at δ(C) 20.9

Table 1. *^{1}H- and ^{13}C-NMR Data, HMBC, and NOESY Correlations of* **1** (in $CDCl_3$)

Position	δ(H)	δ(C)	HMBC	NOESY
1	3.58 (*dd*, *J* = 11.7, 3.8)	75.8	14	3, 2a, 2b, 5
2	2.02–2.07 (*m*, H_a), 1.68–1.74 (*m*, H_b)	33.5	1, 3	1, 2b, 3, 7 1, 2a, 3
3	4.82 (*dd*, *J* = 12.5, 4.7)	75.8	2, 4, 15, Me*C*O	1, 2a, 2b, 5
4	–	73.5		
5	1.67–1.75 (*m*)	54.4	1, 3, 4, 6, 7, 10, 14, 15	1, 3, 7
6	4.38 (*dd*, *J* = 11.1, 5.4)	78.8	5, 8, 10, 12	13, 15, 14
7	2.12–2.19 (*m*)	49.1		2a, 5, 11
8	1.70–1.76 (*m*, H_a), 1.58–1.65 (*m*, H_b)	20.5		
9	1.97–2.04 (*m*, H_a), 1.18–1.25 (*m*, H_b)	39.2		
10	–	41.9		
11	2.57–2.65 (*m*)	37.5		7
12	–	179.1		
13	1.20 (*d*, *J* = 7.7)	9.7	12, 11, 7	6, 11
14	1.00 (*s*)	13.6	5, 1, 10, 9	6
15	1.38 (*s*)	19.0	5, 4, 3	6
AcO	2.09 (*s*)	21.8, 170.8		
OH	3.05 (*s*)			

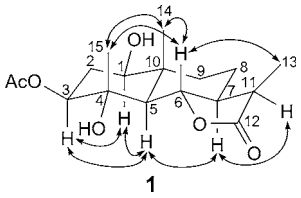

Fig. 2. *The key NOESY* (H ↔ H) *correlations of compound* **1**

Table 2. *^{1}H- and ^{13}C-NMR Data, HMBC, and NOESY Correlations of* **2** (in $CDCl_3$)

Position	δ(H)	δ(C)	HMBC	NOESY
1	3.59 (*dd*, *J* = 11.7, 4.3)	75.7	2, 10, 14	2a, 3, 5, 9b
2	2.18–2.24 (*m*, H_a), 1.57–1.62 (*m*, H_b)	36.9	1, 3, 4, 10	1, 2b, 3 2a, 14
3	5.12–5.19 (*m*)	70.1	Me*C*O	1, 2a, 5
4	–	140.5		
5	2.00–2.06 (*m*)	50.3	4, 6, 10, 14	1, 3
6	4.30 (*dd*, *J* = 10.9, 4.1)	77.3	7, 8	13, 14, 15b
7	2.10–2.15 (*m*)	48.1		11
8	1.68–1.75 (*m*, H_a), 1.51–1.58 (*m*, H_b)	20.1	7, 9	9a, 9b, 8b 7, 8a
9	2.02–2.08 (*m*, H_a),1.27–1.33 (*m*, H_b)	35.7	8, 10 1, 7, 8, 10, 14	8a, 8b, 9b 1, 8a, 9a
10	–	42.4		
11	2.61–2.67 (*m*)	38.5	6, 7, 12, 13	7, 13
12	–	179.6		
13	1.20 (*d*, *J* = 7.6)	9.5	7, 11, 12	6, 11
14	0.83 (*s*)	11.5	1, 5, 9, 10	2b, 6, 9a
15	5.17 (*s*, H_a), 4.99 (*s*, H_b)	107.8	3 5	15b 6, 15a
AcO	2.12 (*s*)	20.9, 169.6		

and 169.6 in the ^{13}C-NMR spectrum. The *singlet*s at δ(H) 5.17 (*s*) and 4.99 (*s*) confirmed the presence of an exomethylene group. A typical *doublet* of *doublet* for H–C(6) at δ(H) 4.30 (*dd*), which correlated with the C-atom at δ(C) 77.3 in the HSQC spectrum, indicated that **2** was also a C(6)-lactonized sesquiterpene lactone. On comparison of NMR spectra data of **2** and **1**, an exomethylene group in **2** replaced a OH and a Me group at C(4) in **1**. The chemical shift of C(13) at δ(C) 9.5 and NOESY correlations observed H–C(6)/Me(13,14) and H_b–C(15), and H–C(7)/H–C(11) let us assign the β-configuration of Me(13) as in **1**. Taking all these data into accountment, compound **2** was characterized as 3β-acetoxy-1β-hydroxy-11α*H*-eudesman-4(15)-en-12,6α-olide, as depicted in *Fig. 1*.

Conclusions. – In conclusion, two new eudesmanolides, 3β-acetoxy-1β,4α-dihydroxy-11α*H*-eudesman-12,6α-olide (**1**) and 3β-acetoxy-1β-hydroxy-11α*H*-eudesman-4(15)-en-12,6α-olide (**2**), were isolated from the flowers of *Achillea millefolium*.

Experimental Part

General. NMR Spectra: *Varian Inova 500NB* NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *VG-ZAB-HS* spectrometer; in *m*/*z*.

Plant Material. The flowers of *A. millefolium* were purchased in September 2003 in Montreal, Quebec, Canada. The plant material was identified by Dr. *J.-H. Wang*, laboratory of Pharmacognosy, Hebei Medical University. Several specimens (under accession voucher number Qw-1999-01) have been deposited with our laboratory, Hebei Medical University.

Extraction and Isolation. Powered air-dried flowers of *A. millefolium* (4.2 kg) were extracted with 16 l of MeOH by shaking for 1 d at r.t. The ground plants were filtered and extracted again with fresh solvent for another 3 d, each with 6 l of MeOH. The combined org. extract was evaporated under reduced pressure. H_2O (4 l) was added to the crude extract, and lipids were removed by stirring the mixture with hexane (4×3.2 l). The aq. phase was then salted with NaCl and extracted with AcOEt for four times (each 3.2 l). The combined AcOEt extracts were dried (anh. Na_2SO_4), filtered, and evaporated in a rotary evaporator to yield a dark brown extract of 149 g.

The AcOEt extract was subjected to column chromatography (CC) on silica gel *60* (SiO_2; 60–200 mesh, 1.5 kg, 8×68 cm), eluted with AcOEt/MeOH 95:5 (2.5 l). After elution, the SiO_2 was cut into 15 equal bands, and each band was individually eluted with AcOEt/MeOH 1:1. The relents of the column from bands 3 to 6 were combined after analyses by TLCs and evaporated to yield 43 g of residue *A*, which then was fractionated by CC on SiO_2 (1200 g, 9.5×30) with hexane/AcOEt (3:1 to 1:4) as eluent. The eluted fractions were monitored by TLC and combined into ten pooled fractions (*Frs.* A_1–A_{10}). *Fr.* A_3 was further fractionated by SiO_2 CC. Elution was carried out using gradients of hexane/acetone (2:1 to 1:1). Combination of the fractions on the basis of TLC analyses afforded eight fractions (*Frs.* $A_{3\text{-}1}$–$A_{3\text{-}8}$). *A* part of *Fr.* $A_{3\text{-}3}$ was submitted to prep. TLC with hexane/AcOEt 3:2 as developing solvent to yield *Frs.* $A_{3\text{-}3\text{-}1}$ and $A_{3\text{-}3\text{-}2}$. Both of them were further purified by prep. TLC developed with hexane/acetone (5:2) and yielded **1** (1.5 mg, R_f 0.38). A part of *Fr.* $A_{3\text{-}6}$ was submitted to prep. TLC with hexane/AcOEt 1:1 as developing solvent to yield *Frs.* $A_{3\text{-}6\text{-}1}$ and $A_{3\text{-}6\text{-}2}$. *Fr.* $A_{3\text{-}6\text{-}1}$ was further purified by prep. TLC, developed with hexane/acetone 3:2, and yielded **2** (1.3 mg, R_f 0.33).

*3β-Acetoxy-1β,4α-dihydroxy-11α*H*-eudesman-12,6α-olide* (=*8-(Acetyloxy)decahydro-6,9-dihydroxy-3,5a,9-trimethylnaphtho[1,2-*b*]furan-2(3*H*)-one*; **1**). Gum. $[\alpha]_D^{22} = +23$ ($c = 0.10$, MeOH). ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-FAB-MS: 365.1360 ($[M+K]^+$; calc. 365.1366).

*3β-Acetoxy-1β-hydroxy-11α*H*-eudesman-4(15)-en-12,6α-olide* (=*8-(Acetyloxy)decahydro-6-hydroxy-3,5a-dimethyl-9-methylenenaphtho[1,2-*b*]furan-2(3*H*)-one*; **2**). Gum. $[\alpha]_D^{22} = +31$ ($c = 0.10$, MeOH). ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-FAB-MS: 347.1255 ($[M+K]^+$; calc. 347.1261).

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