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DITERPENES FROM *ACHILLEA CLYPEOLATA*

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Key Word Index—*Achillea clypeolata*; Asteraceae; diterpenes; 16α,17-epoxy-*ent*-kaurane; 19-acetoxy-16α,17-epoxy-*ent*-kaurane; 3α-acetoxy-16α,17-epoxy-*ent*-kaurane.

Abstract—The isolation of 16α,17-epoxy-*ent*-kaurane (a known compound), 3α-acetoxy-16α,17-epoxy-*ent*-kaurane and 19-acetoxy-16α,17-epoxy-ent-kaurane from roots of *Achillea clypeolata* is reported. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In our previous studies on the Yugoslavian species of *Achillea* [1–4], a number of sesquiterpene lactones, mostly guaianolides, have been isolated from the aerial parts of the plants studied. In a continuation of these studies, we have now examined extracts of the aerial parts and roots of *A. clypeolata* Sibth. et Sm., extracted according to the usual procedures used for the isolation of sesquiterpene lactones [5] and alkamides [6], respectively.

RESULTS AND DISCUSSION

Silica gel column chromatography of the extracts afforded diterpenes **1–3** (roots) and a mixture of β-sitosterol and stigmasterol (both extracts). Neither the expected sesquiterpene lactones, nor the alkamides (the usual constituents of the roots of *Achillea* species [7]) were isolated from the extracts. The ^{1}H and ^{13}C NMR data for compounds **1–3** are present in Tables 1 and 2. The assignment of diterpene **1** as 16α,17-epoxy-*ent*-kaurane was based on a comparison of its spectral data and optical rotation to those reported for the same compound originating from the aerial parts of *Baccharia minutiflora* [8]. The ^{1}H NMR spectral assignments of the angular methyls in **1**, according to heteronuclear one-bond C,H-correlation (HMQC), were different from the original ones [8]. According to HMQC, the previous assignment of a signal at δ 2.04 as H-15 [8] should be altered to H-14.

Isomers **2** and **3**, both exhibiting a molecular ion at m/z 346 ($C_{22}H_{34}O_3$) and an acetate carbonyl IR band (*ca* 1730 cm^{-1}), were identified as 19- and 3α-acetoxy-16α,17-epoxy-*ent*-kauranes, respectively. The chemical shifts and coupling pattern of the distereotopic 19-methylene in **2** (see Table 1) were analogous to those observed in the previously identified 19-acetoxy-*ent*-kaurene [9], as well as in 19-acetoxy-15β,16β-epoxy-*ent*-beyeran [10], a diterpene with the same stereostructure of the A/B rings as the *ent*-kaurane series. It should also be noted that among the co-occurring diterpenes of **1** in *B. minutiflora*, the corresponding 19-alcohol (**4**) was reported [8]. The 3α-acetoxy structure in **3** was in agreement with the following ^{1}H and ^{13}C NMR evidence: (i) occurrence of a one proton *dd* (δ 4.46, $J_{ae} = 5.1$ and $J_{aa} = 11.2$ Hz) in the ^{1}H NMR spectrum, dipolarly coupled with the syn-axial H-1β (δ 0.98) (NOESY) and assigned as axial H-3β α-positioned to the acetoxy group, and (ii) the downfield shifts of C-3, C-2 and C-4 ($\Delta\delta$ 39.0, 5.0 and 4.5, respectively) and upfield shifts of C-18, C-19 and C-1 ($\Delta\delta - 5.3$, -4.9 and -2.0, respectively) compared to those of the same carbons in **1**.

EXPERIMENTAL

Plant material. Achillea clypeolata (voucher 0594 deposited at the Department of Chemistry, Faculty of Philosophy, University of Niš, Yugoslavia) was collected at Sićevačka Klisura (southeast Serbia) during the flowering season at the end of May 1994.

Isolation procedure. Crude extracts of air-dried aerial parts (1 kg) and roots (0.6 kg) were obtained by extraction with Et_2O–petrol–MeOH (1 : 1 : 1) [5] and Et_2O–petrol (1 : 2) [6], respectively.

Roots. The crude extract (3.4 g) was subjected to CC on silica gel, starting elution with petrol and gradually

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Table 1. Partial ^1H NMR (600 MHz, $CDCl_3$, TMS as int. standard) data for compounds **1–3**

H	**1***	**2**	**3***
1α	1.81 *dt* (13.0, <3.5)	1.87 *dt* (13.5, <3.5)	1.85 *dt* (1.33, <3.5)
1β	0.75 *dt* (3.5, 13.0)	0.86 *dt* (4.0, 13.5)	0.98 *dt* (4.3, 13.3)
3α	*ca* 1.38†	‡	—
3β	1.12 *dt* (4.7, 14.2)	0.99 *dt* (4.1, 13.7)	4.46 *dd* (5.1, 11.2)
5	0.78 *br d* (12.6)	‡	*ca* 0.83†
9	1.06 *d* (7.4)	1.08 *d* (7.4)	*ca* 1.05†
13	1.78 *m* (Wh/2 12)	1.80 *m* (Wh/2 12)	1.80 *m* (Wh/2 12)
14	2.04 *dd* (11.7, 1.7)	2.00 *dd* (11.5, 1.9)	2.02 *dd* (12.1, 1.6)
14′	*ca* 1.44	‡	*ca* 1.43†
17	2.79 *d* (4.7)	2.80 *d* (4.7)	2.79 *d* (4.6)
17′	2.86 *d* (4.7)	2.87 *d* (4.7)	2.87 *d* (4.6)
18	0.85 *s*	0.95 *s*	0.86 *s*
19	0.80 *s*	3.88 *d* (1.1) 4.22 *d* (11.1)	0.86 *s*
20	1.03 *s*	1.05 *s*	1.06 *s*
OAc	—	2.05 *s*	2.04 *s*

*Assigned by means of HMQC, COSY and NOESY.

†Overlapped with other signals.

‡Overlapped with other signals, could not be assigned.

increasing the polarity by addition of Et_2O. Diterpene **1** (200 mg) was isolated from fr. 1 (240 mg), petrol–Et_2O (9:1), after repeated CC (same conditions as above). Fr. II (450 mg), petrol–Et_2O (17:3), purified by repeated CC (same conditions as above), followed by prep. TLC (Kieselgel 60 GF_{254}, layer thickness 1 mm, petrol–Et_2O, 4:1, 3 developments) yielded **2** (1.3 mg), Fr. III (130 mg), petrol–Et_2O (4:1), after repeated CC (same conditions as above) afforded **3** (83 mg). Fr. IV (20 mg), petrol–Et_2O (7:3), contained β-sitosterol and stigmasterol in the ratio of *ca* 10:7.

Table 2. ^{13}C NMR (75 MHz, $CDCl_3$, TMS as int. standard) data for compounds **1–3**

C	**1***	**2†**	**3***
1	40.4	40.4	38.4
2	18.6	18.2	23.6
3	42.0	36.4	81.0
4	33.2	37.1	37.7
5	56.2	56.8‡	55.2
6	20.2	20.4	19.8
7	41.1	41.4	40.9
8	45.4	45.3	45.1
9	55.9	56.1‡	55.6
10	39.3	39.6	38.9
11	19.3	19.4	19.4
12	29.2	29.1	29.1
13	42.7	42.7	42.6
14	38.6	38.4	38.5
15	48.9	48.8	48.7
16	66.4	66.4	66.3
17	50.4	50.5	50.4
18	33.6	27.6	28.3
19	21.6	67.2	16.7
20	17.8	18.0	17.8
AcO	—	21.1 171.5	21.3 171.0

*Assigned by means of DEPT, HMQC and comparison of chemical shifts with those of *ent*-kaurene [11].

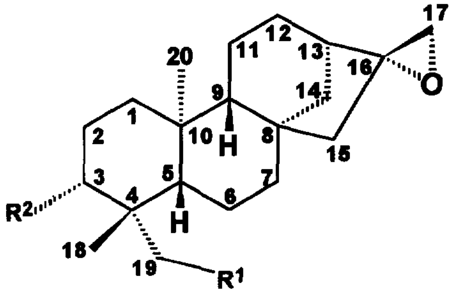
†Assigned by means of DEPT and comparison of chemical shifts with those of *ent*-kaurene [11].

‡The assignments can be interchanged.

Aerial parts. A MeOH-soluble portion (25 g) of the crude extract was subjected to CC on silica gel, using an analogous procedure to the one described above to yield 18 mg of a mixt. of β-sitosterol and stigmasterol (*ca* 5:2).

*16α,17-Epoxy-*ent*-kaurane* (**1**). Mp 115° (Me_2CO), $[\alpha]_D^{18}$ −55° ($CHCl_3$; *c* 0.09), lit. [8] $[\alpha]_D^{24}$ −51° ($CHCl_3$; *c* 1.2). EIMS (probe) 70 eV, *m/z* (rel. int.): 288 $[M]^+$ (100), 273 $[M-15]^+$ (45), 255 (22), 216 (42), 201 (23), 137 (37), 133 (44), 123 (78), 109 (49). IR ν_{max}^{film} cm^{-1}: 1460, 1387, 1368, 971, 953, 907; ^1H and ^{13}C NMR: Tables 1 and 2.

*16α,17-Epoxy-19-acetoxy-*ent*-kaurane* (**2**). Mp 63–66°, $[\alpha]_D^{18}$ −74° ($CHCl_3$; *c* 0.07). EIMS (probe) 70 eV, *m/z* (rel. int.): 346 $[M]^+$ (88), 331 $[M-15]^+$ (7), 286



1: $R^1 = R^2 = H$

2: $R^1 = OAc$, $R^2 = H$

3: $R^1 = H$, $R^2 = OAc$

4: $R^1 = OH$, $R^2 = H$ [8]

$[M-60]^+$ (41), 274 (44), 273 (58), 255 (41), 135 (58), 123 (77), 109 (70), 43 (100); IR ν_{max}^{film} cm^{-1}: 1733, 1482, 1463, 1445, 1386, 1236; ^{1}H and ^{13}C NMR: Tables 1 and 2.

*16α,17-Epoxy-3α-acetoxy-*ent*-kaurane* (**3**). Mp 106–110°, $[\alpha]_D^{18}$ $-70°$ ($CHCl_3$; *c* 0.07). EIMS (probe) 70 eV, *m/z* (rel. int.): 346 $[M]^+$ (11), 286 $[M-60]^+$ (12), 271 (8), 135 (16), 121 (19), 95 (14), 93 (22), 91 (30), 43 (100); IR ν_{max}^{film} cm^{-1}: 1729, 1482, 1462, 1450, 1395, 1368, 1249; ^{1}H and ^{13}C NMR: Tables 1 and 2.

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