The only difference was the use of Et_2O rather than petrol for oil separation from the aq. seed extract.

Kinetic experiments were carried out at 30° and pH 4.20 as follows: 2.0 ml of the acid lipase soln (1.0 mg/ml, 10 mM Tris buffer, pH 8.1) was mixed with 2.0 ml of the substrate emulsion (0.50 mmol/ml in 5% aq. gum arabic). Enough 0.1 M HOAc was then added to adjust the pH to 4.20. The amount of free acid generated was followed by potentiometric titration with 50 mM NaOH as described in ref. [8]. Initial reaction rates were determined according to Boeker [9] as:

$$v_i = \lim_{P \to 0} \Delta P/\Delta t.$$

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FURTHER SILPHINENE DERIVATIVES FROM *CINERARIA GEIFOLIA* VAR. *GLABRA*

J. JAKUPOVIC, W.-R. ABRAHAM and F. BOHLMANN

Institute for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, West Germany

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Key Word Index—*Cineraria geifolia* var. *glabra*; Compositae; sesquiterpenes; silphinene derivatives.

Abstract—A reinvestigation of the sesquiterpenes from the aerial parts of *Cineraria geifolia* var. *glabra* afforded eight new silphinene derivatives. One of these compounds had been isolated previously but its structure had not been elucidated with certainty. The stereochemistry of a corresponding isovalerate from *Callilepis salicifolia* has to be revised.

INTRODUCTION

A keto diester had been isolated from *Cineraria geifolia var. glabra* (tribe Senecioneae) [1]. However, the decision between two possible structures was difficult and therefore only a preliminary structure was reported [1]. A reinvestigation of this compound and some minor related sesquiterpenes allowed a clear assignment of the structures. In addition to the acetoxy angelate **1**, which was isolated previously, three further diesters (**2–4**), the hydroxy ester **5** and the monoesters **6–8** were obtained.

RESULTS AND DISCUSSION

The ^{1}H NMR spectral data of **1** (Table 1) were close to those of a ketone obtained by oxidation of a hydroxy isovalerate from *Callilepis salicifolia* [2]. Careful spin decoupling allowed the assignment of all signals. The resulting sequences of the carbons C-1–C-3 and C-7, C-11, C-10–C-9 (C-15) showed that we were dealing with a derivative of silphinene [3]. The stereochemistry, however, could only be established by NOE difference spectroscopy (Table 2). Also the relative position of the ester group could be assigned from the NOEs. Thus irradiation of H-12 caused a clear effect with the methyl signals of the angelate residue, while H-13 and H-14 showed NOEs with the acetoxy methyl. Furthermore, inspection of a model indicated that due to the quasi-axial orientation of the 11β-acetoxy group the H-5 signal was shifted downfield. As H-11 showed a clear NOE with H-7 and H-10α the β-orientation of the acetoxy group was established. The assignment of H-10α followed from the observed small coupling with H-9. As H-15 showed an NOE with H-1 the α-orientation of H-9 was settled. Similarly all other orientations followed from the observed NOEs. The ^{13}C NMR signals (see Experimental) also agreed nicely with the proposed structure.

The ^{1}H NMR spectra of **2–4** (Table 1) were nearly identical with that of **1**, only the typical signal of the

Table 1. ^{1}H NMR spectral data of 1–9 (400 MHz, $CDCl_3$, TMS as int. standard)

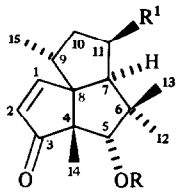
H	1	2	3	4	5	6	7	8	9
1	7.57 *d*		7.57 *d*		7.56 *d*	7.58 *d*	7.57 *d*		7.56 *d*
2	6.12 *d*		6.12 *d*		6.09 *d*	6.01 *d*	6.01 *d*		6.00 *d*
5	5.43 *s*		5.33 *s*		5.45 *s*	5.15 *s*	5.03 *s*		5.04 *s*
7	2.22 *d*	2.23 *d*	2.21 *d*		2.04 *d*	2.14 *dd*	2.10 *dd*		2.11 *dd*
9	2.62 *ddq*		2.62 *ddq*		2.82 *ddq*	2.19 *ddq*	2.18 *ddq*	2.19 *ddq*	2.19 *ddq*
10α	1.57 *ddd*		1.56 *ddd*		1.56 *ddd*	1.34 *dddd*	1.35 *m*		1.34 *dddd*
10β	2.10 *dd(br)*		2.09 *dd(br)*		1.92 *dd(br)*	1.95 *m*	1.95 *m*		1.94 *ddd(br)*
11α	5.25 *dd(br)*		5.23 *dd(br)*		4.36 *dd(br)*	1.80 *m*	1.79 *m*		1.78 *ddd(br)*
11β	—		—		—	1.43 *dddd*	1.40 *m*		1.42 *dddd*
12	0.90 *s*	0.89 *s*	0.87 *s*		0.87 *s*	0.84 *s*	0.81 *s*		0.82 *s*
13	0.94 *s*	0.93 *s*	0.92 *s*		1.16 *s*	0.94 *s*	0.91 *s*	0.90 *s*	0.91 *s*
14	1.25 *s*	1.22 *s*	1.21 *s*	1.22 *s*	1.24 *s*	1.20 *s*	1.17 *s*	1.16 *s*	1.16 *s*
15	0.92 *d*		0.92 *d*		0.92 *d*	0.92 *d*	0.91 *d*		0.92 *d*
OAc	2.17 *s*	2.18 *s*	2.15 *s*		—	—	—	2.13 *s*	—
OR	6.11 *qq*	6.99 *dq*	2.67 *qq*	2.49 *dq*	6.09 *qq*	6.10 *qq*	2.48 *dq*		2.32 *dd*
	2.04 *dq*	1.83 *dq*	1.26 *d*	2.40 *dq*	2.03 *dq*	2.03 *dq*	2.40 *dq*		2.27 *dd*
	1.94 *dq*	1.88 *dq*	1.23 *d*	1.19 *t*	1.94 *dq*	1.94 *dq*	1.19 *t*		2.17 *ddqq*
									1.00 *d*
									0.99 *d*

J (Hz): Compounds **1**–**5**: 1, 2 = 5.5; 7, 11 = 4; 9, 10α = 10α, 10β = 12.5; 9, 10β = 6; 9, 15 = 7; 10α, 11 = 3; compounds **6**–**9**: 1, 2 = 5.5; 7, 11α = 8; 7, 11β = 9, 10α = 10α, 10β = 12; 9, 10β = 10β, 11β = 6; 9, 15 = 10α, 11α = 7; 10α, 11β = 5; 11α, 11β = 12.5; OAng, OTigl: 3′, 4′ = 7; 3′, 5′ = 4′, 5′ = 1.5; OiBu: 2′, 3′ = 2′, 4′ = 7; OProp: $2_1'$, $2_2'$ = 16.5; 2, 3 = 7.

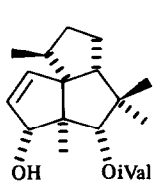
changed ester residue being different. A small upfield shift of the H-5 signal in the isobutyrate **3** and the propionate **4** is characteristic for saturated esters if compared with the shift of unsaturated ones. This also allowed the assignment of the relative position of the ester group in **2**, while biogenetic considerations led to the assumption that in **3** and **4** the *O*-acetate group also was at C-11. This was supported by the fact that in all cases (also with **5**–**9**, see below) a strong MS fragment was observed formed by loss of RCO, which most likely was due to a neighbouring group effect of the cyclopentenone system.

The ^{1}H NMR spectrum of **5** (Table 1) only differed in the chemical shift of H-11 from that of **1**. Accordingly, the corresponding desacetyl derivative was present.

The ^{1}H NMR spectra of **6**–**8** (Table 1) were nearly identical with that of the already mentioned isovalerate (**9**) obtained by oxidation of the *Callilepis* carbinol [2]. Again all signals could be assigned by spin decoupling and the



	1	2	3	4	5	6	7	8	9
R	Ang	Tigl	iBu	Prop	Ang	Ang	Prop	Ac	iVal
R^1	OAc	OAc	OAc	OAc	OH	H	H	H	H



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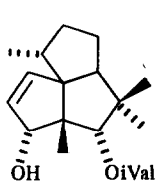

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Table 2. NOE effects with **1** and **9**

Irradiation of	NOE observed (effects in % in parentheses)
	1
H-12	H-1 (2); H-2 (2); H-7 (10); OAng: H-4′ (1); H-5′ (1)
H-15	H-1 (6); H-10β (2)
H-13	H-5 (8); H-11 (6); OAc (2)
H-14	H-5 (12); H-9 (14); OAc (2)
H-10α	H-11 (5); H-7 (3)
H-10β	H-9 (6)
H-7	H-1 (5); H-11 (10); H-10β (3); H-12 (5)
H-9	H-10β (6); H-14 (5)
H-11	H-7 (10); H-10α (4); H-13 (4)
H-5	H-13 (4); H-14 (5)
	9
H-12	H-1 (2); H-2 (2); H-7 (10); H-13 (10)
H-13	H-5 (6); H-11 (4); H-12 (6); H-14 (4)
H-14	H-5 (7); H-9 (10); H-13 (4)
H-15	H-1 (6)

stereochemistry was established by NOE difference spectroscopy. In the case of **9**, especially clear NOEs between H-14 and H-5 and between H-13 and H-5 showed that the configuration at C-5 was the same as in **1**–**5**. The same effects were obtained with the esters **6**–**8**. Thus the stereochemistry of all the monoesters was settled. This required a revision of the configuration of the silphinene derivative from *Callilepis salicifolia* (**10**) to **11**, the epimer at C-3 and C-5. Accordingly, reduction of **9** gave the natural compound. As followed from inspection of a model, the hydride attack is probably directed by the configuration of the ester group at C-5. From the observed Cotton-effect of **9** the opposite absolute configuration was deduced. Biogenetic considerations, however, showed that probably all these triquinanes are derived from (−)-caryophyllene [3] indicating that the presented absolute configuration is more likely. This is supported by the fact that (−)-caryophyllene occurs together with all the known sesquiterpenes of this type [3]. In one case this could be established by synthesis of (−)-silphiperfol-6-ene starting with (*R*)-(+)-pulegone [4].

EXPERIMENTAL

The extract (Et_2O–petrol, 1:2) of the air dried aerial parts (10 g, collected on Table Mountain in September 1977, voucher 77/337) was separated in the usual fashion [5]. TLC (silica gel PF 254, Et_2O–petrol–$CHCl_3$, 1:7:2, four developments) gave 2 mg **6** (R_f 0.73, values always for four developments), 3 mg **7** (R_f 0.64), 3 mg **8** (R_f 0.60), 15 mg **1** (R_f 0.38), 2 mg **2** (R_f 0.34), 2 mg **3** (R_f 0.32), 5 mg **4** (R_f 0.27) and 2 mg **5** (R_f 0.20). Though all compounds were homogeneous by TLC in different solvent mixtures and by 400 MHz ^{1}H NMR spectroscopy they could not be induced to crystallize and they were obtained as colourless oils.

11β-Acetoxy-5α-angeloyloxy-silphinen-3-one (**1**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745 (OAc), 1715 (C=CCO$_2$R, C=CC=O); MS *m/z* (rel. int.): 374.209 [M]$^+$ (5) (calc. for $C_{22}H_{30}O_5$: 374.209), 291 [M − COR]$^+$ (32), 274 [M − RCO$_2$H]$^+$ (12), 232 [274 − ketene]$^+$ (5), 214 [274 − HOAc]$^+$ (20), 83 [C_4H_7CO]$^+$ (100), 55 [83 − CO]$^+$ (62), 43 [MeCO]$^+$ (42); ^{13}C NMR ($CDCl_3$, C-1–C-11): 167.6 *d*, 138.3 *d*, 211.0 *s*, 64.1 *s*, 86.4 *d*, 42.6 *s*, 61.9 *d*, 57.2 *s*, 35.2 *d*, 41.9 *t*, 76.2 *d*; OAng: 168.0 *s*, 128.2 *s*, 131.9 *d*; OAc: 170.0 *s* (methyl signals: 26.6 *q*, 23.8 *q*, 21.7 *q*, 20.6 *q*, 19.9 *q*, 15.9 *q*, 15.8 *q*);

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589 \quad 578 \quad 546 \quad 436\,\text{nm}}{-69 \quad -72 \quad -85 \quad -184} \ (CHCl_3;\ c = 0.7).$$

11β-Acetoxy-5α-tigloyloxy-silphinen-3-one (**2**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745 (OAc), 1715 (C=CCO$_2$R, C=CC=O); MS *m/z* (rel. int.): 374.209 [M]$^+$ (3) (calc. for $C_{22}H_{30}O_5$: 374.209), 291 [M − COR]$^+$ (28), 275 [M − OCOR]$^+$ (10), 215 [275 − HOAc]$^+$ (22), 83 [C_4H_7CO]$^+$ (100).

11β-Acetoxy-5α-isobutyryloxy-silphinen-3-one (**3**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740 (OAc, CO$_2$R), 1715 (C=CC=O); MS *m/z* (rel. int.): 362.209 [M]$^+$ (1) (calc. for $C_{21}H_{30}O_5$: 362.209), 291 [M − COR]$^+$ (30), 275 [M − OCOR]$^+$ (9), 215 [275 − HOAc]$^+$ (21), 71 [C_3H_7CO]$^+$ (30), 43 [71 − CO]$^+$ (100).

11β-Acetoxy-5α-propionyloxy-silphinen-3-one (**4**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740 (OAc, CO$_2$R), 1715 (C=CC=O); MS *m/z* (rel. int.): 348.194 [M]$^+$ (10) (calc. for $C_{20}H_{28}O_5$: 348.194), 292 [M − O=C=CHMe]$^+$ (42), 291 [M − COEt]$^+$ (85), 250 [292 − ketene]$^+$ (27), 231 [291 − HOAc]$^+$ (37), 57 [C_2H_5CO]$^+$ (100).

11β-Hydroxy-5α-angeloyloxy-silphinen-3-one (**5**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1715 (C=CCO$_2$R, C=CC=O); MS *m/z* (rel. int.): 332.199 [M]$^+$ (1) (calc. for $C_{20}H_{28}O_4$: 332.199), 249 [M − COR]$^+$ (21), 233 [M − OCOR]$^+$ (11), 215 [233 − H_2O]$^+$ (37), 83 [C_4H_7CO]$^+$ (100), 55 [83 − CO]$^+$ (67).

5α-Angeloyloxy-silphinen-3-one (**6**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1715 (C=CCO$_2$R, C=CC=O); MS *m/z* (rel. int.): 316.204 [M]$^+$ (10) (calc. for $C_{20}H_{28}O_3$: 316.204), 233 [M − COR]$^+$ (90), 217 [M − OCOR]$^+$ (55), 83 [C_4H_7CO]$^+$ (100).

5α-Propionyloxy-silphinen-3-one (**7**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740 (CO$_2$R), 1715 (C=CC=O); MS *m/z* (rel. int.): 290.188 [M]$^+$ (20) (calc. for $C_{18}H_{26}O_3$: 290.188), 233 [M − COR]$^+$ (100), 217 [M − OCOEt]$^+$ (60), 57 [EtCO]$^+$ (70).

5α-Acetoxy-silphinen-3-one (**8**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745 (OAc), 1715 (C=CC=O); MS *m/z* (rel. int.): 276.172 [M]$^+$ (17) (calc. for $C_{17}H_{24}O_3$: 276.172), 233 [M − COMe]$^+$ (100), 217 [M − OAc]$^+$ (65).

Boranate reduction of 5α-isovaleryloxy-silphinen-3-one. To 5 mg **9** in 2 ml MeOH, 10 mg $NaBH_4$ was added. After 15 min. dil H_2SO_4 was added. TLC (Et_2O–petrol, 1:1) of the reaction product gave 3 mg 3α-hydroxy-5α-isovaleryloxy-silphinene, identical with the natural compound by co-TLC and ^{1}H NMR.

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