Helichrysum heterolasium *Hilliard* (*Herbar Nr.* 77/280): 50 g Wurzeln ergaben 0.3 mg **1** und 1 mg **3**, während 210 g oberirdische Teile 44 mg **3**, 3 mg **5**, 3 mg **6**, 1.1 g **7**, 78 mg **8**, 145 mg **9**, 82 mg **10**, 16 mg **11**, 8 mg **12**, 42 mg **13**, 30 mg **14** (Ether/Petrol 1:10), 47 mg **21** (Ether) und 40 mg **22** lieferten.

14,15-*Dihydrohelicallen*-16-*säure* (**16**). Farbloses Öl, IR: CO_2H 3500–2600, 1705 cm^{-1}. MS: M^+ *m/e* 304.240 (37%) (ber. für $C_{20}H_{32}O_2$ 304.240); $-{}^{\cdot}Me$ 289 (2); −Isopren 236 (10) $C_7H_9^+$ 93 (100).

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589}{+198.6}\ \frac{578}{+207.7}\ \frac{546\text{ nm}}{+235.5}\ (c = 3.4).$$

20 mg **16** wurden mit Diazomethan verestert. Nach DC (Ether/Petrol 1:10) erhielt man 19 mg **17**, ^{1}H-NMR s. Tabelle 1.

Helicallen-16-*al* (**18**). Farbloses Öl, IR: C=CCHO 2720, 1690, 1650 cm^{-1}. MS: M^+ *m/e* 286.230 (13%) (ber. für $C_{20}H_{30}O$ 286.230); $-C_5H_8O$ (McLafferty) 202 (33); C_7H_9 93 (100).

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589}{+16.0}\ \frac{578}{+18.0}\ \frac{546\text{ nm}}{+23.3}\ (c = 1.0).$$

10 mg **18** reduzierte man in MeOH mit $NaBH_4$. Nach DC (Ether/Petrol 1:3) erhielt man 7 mg **19**, identisch mit dem Naturstoff.

Helicallen-16-*ol* (**19**). Farbloses Öl, IR: OH 3615 cm^{-1}. MS: M^+ *m/e* 288.245 (3%) (ber. für $C_{20}H_{32}O$ 288.245); $-H_2O$ 270 (7); $-C_5H_{10}O$ 202 (89) (McLafferty); $C_7H_9^+$ 93 (100).

1-*Myrtenolacetat* (**14**). Farbloses Öl, IR: OAc 1740, 1240; C=C 3030, 1650 cm^{-1}. MS: M^+ *m/e* −; −Keten 152 (5); −AcOH 134 (14); 134 $-{}^{\cdot}Me$ 119 (38); $C_7H_7^+$ 91 (100); $MeCO^+$ 43 (42). ^{1}H-NMR: *dddd* 5.57 (2-H); *d*(*br*) 2.34 (3-H); *d*(*br*) 2.25 (3′-H); (*dbr*) 2.13 (4-H); *ddd* 2.42 (5-H); *d* 1.20 (5′-H); *d*(*br*) 2.13 (6-H); *dddd* 4.42 (7-H); *dddd* 4.49 (7′-H); *s* 1.34 (9-H); *s* 0.84 (10-H); *s* 2.06 (OAc) [*J* (Hz): 2,3 = 2.5; 3,3′ = 1.5; 2,6 = 2,7 = 2,7′ = 1.5; 3,3′ = 16; 3,5 = 5.5; 5,5′ = 8.5; 5,6 = 5.5; 7,7′ = 13].

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589}{-40.4}\ \frac{578}{-42.0}\ \frac{546\text{ nm}}{-47.8}\ (c = 0.46).$$

2,4,4′-*Trihydroxy*-6-*methoxychalkon* (**21**). Gelbgefärbtes Öl, IR: OH 3500–2600; C=O 1650 cm^{-1}. MS: M^+ *m/e* 286.084 (95%) (ber. für $C_{16}H_{14}O_5$ 286.084); $-{}^{\cdot}H$ 285 (100); $-{}^{\cdot}C_6H_4OH$ 193 (41); $-{}^{\cdot}CH{=}CHC_6H_4OH$ 167 (82). UV (Ether) λ_{max} 347 nm.

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MARGOTIANIN, A NEW DITERPENOID FROM *MARGOTIA GUMMIFERA*

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Key Word Index—*Margotia gummifera* (= *Elaeoselinum gummiferum*); Umbelliferae; diterpenoid; *ent*-atis-16-ene derivative; ^{13}C NMR data.

Abstract—From the roots and aerial parts of *Margotia gummifera* a new natural diterpenic methyl ester, margotianin, has been isolated. Its structure was established as methyl *ent*-7α-angeloxy-15α-acetoxy-atis-16-en-19-oate almost exclusively by ^{13}C NMR spectroscopy.

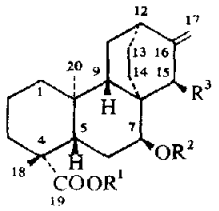
INTRODUCTION

In a previous communication [1], we reported gummiferolic acid (**1**) and *ent*-kaur-16-en-19-oic acid as the major diterpenic constituents of the roots of *Margotia gummifera* (Desf.) Lange [= *Elaeoselinum gummiferum* (Desf.) Tutin]. A study of the less polar fractions of the roots extract and also of the diterpenic compounds extracted from the aerial parts of this plant, has now allowed the isolation of a minor diterpenic constituent, margotianin (**2**), which is a natural methyl ester with the *ent*-atis-16-ene skeleton.

RESULTS AND DISCUSSION

Margotianin (**2**), $C_{28}H_{40}O_6$, had an IR spectrum which showed strong ester (1720, 1695, 1265 cm^{-1}) and exocyclic methylene (3080, 1650, 920 cm^{-1}) absorptions and no −OH bands. The ^{1}H NMR spectrum of compound **2** was very similar to that of gummiferolic acid (**1**) [1] with characteristic signals for an angelic ester axially oriented on a secondary carbon atom, an exocyclic methylene and two methyl groups attached to fully substituted carbon atoms (see Experimental). In addition, margotianin (**2**) showed signals for a methyl

ester (a 3H singlet at δ 3.62) and a secondary acetate group in an allylic position without vicinal protons (3H singlet at δ 1.92 for the acetoxyl and a narrow multiplet, $W_{\frac{1}{2}}$ = 3 Hz. for its geminal proton at δ 5.29).



	R^1	R^2	R^3
1	H	—COCMe=CHMe	H
2	Me	—COCMe=CHMe	OAc
3	Me	—COCMe=CHMe	H
4	Me	H	OH
5	Me	Ac	OAc

By analogy with gummiferolic acid (**1**), all the above data of margotianin may be accommodated on structure **2**, but it is also compatible with an *ent*-kaur-16-ene skeleton. However, this last alternative structure for margotianin must be discarded on the basis of its ^{13}C NMR spectrum, which also provided conclusive proof on the stereochemistry at C-15.

Effectively, C-1, C-2, C-3, C-4, C-5, C-6, C-7, C-10, C-18, C-19 and C-20 carbon resonances of margotianin (**2**, see Table 1) were in complete agreement with those reported for methyl *ent*-7α-acetoxy-atis-16-en-19-oate [2], whereas C-11, C-12, C-13, C-14, C-15, C-16 and C-17 carbon resonances were almost identical to those found in some *ent*-15α hydroxylated *ent*-atis-16-ene alkaloids [3, 4], but different to the previously reported values for 15-hydroxy-*ent*-kaur-16-enes [5]. On the other hand, all the carbon resonances of margotianin (**2**), including the C-8 and C-9 carbon atoms, were in very good agreement (Table 1) with the calculated values obtained from gummiferolic acid methyl ester (**3**) [1] taking into account the introduction of an *ent*-15α-OH on its bicyclo-[2.2.2]-octane system [6, 7] and also the effects caused on the C-8, C-15, C-16 and C-17 carbon atoms by esterification of this allylic alcohol [4, 8]. In particular, the diamagnetic shift ($\Delta\delta$ = −7.6 ppm) experienced by the C-9 carbon atom in compound **2** with respect to **3** clearly pointed to *ent*-α as the configuration of the C-15 ester function. It is remarkable that the small difference between C-20 carbon resonances in compounds **2** and **3** may be due to deformations of the margotianin C and D rings caused by the C-7, C-15 substituent interactions [2].

Finally, the location of the acetate group at C-15 and hence the angeloxy moiety at the C-7 position of margotianin was confirmed as follows. Alkaline hydrolysis of compound **2** under strong conditions (see Experimental) yielded the dihydroxy ester **4**, which was treated with Ac_2O–pyridine to give compound **5**. The ^1H NMR spectrum of **5** showed the signal for the C-15 proton at identical field to that of compound **2** (δ 5.32 and 5.29, respectively), whereas the triplet assigned to the equatorial C-7 proton appeared at higher field with respect to margotianin (diamagnetic shift = − 0.21 ppm). Thus, the angelic ester of the natural diterpenoid is located at C-7.

Table 1. ^{13}C chemical shifts in ppm relative to TMS

Carbon No.	3	2	Calc. for 2 from 3*
1	39.7	40.0	—
2	18.7	18.8	—
3	38.2	38.3	—
4	43.3	43.3	—
5	49.4	49.2	—
6	26.9†	26.6†	—
7	75.7	75.0‡	—
8	36.8	38.9	40.0
9	47.6	40.0	40.1
10	38.0	37.7	—
11	27.9‡	26.5†	27.2
12	36.3	35.5	35.6
13	27.7‡	26.4†	25.8
14	25.5†	26.0†	25.0
15	41.7	74.8‡	~75.0
16	151.1	151.4	150.0
17	105.1	111.3	111.0
18	28.5	28.4	—
19	177.4	177.4	—
20	11.6	12.4	—
1′§	166.9	166.8	—
2′	128.3	128.7	—
3′	136.8	136.1	—
4′	20.9	21.0‖	—
2″	15.8	15.7	—
OMe	51.1	51.2	—
CH_3CO	—	21.6‖	—
CH_3CO	—	170.1	—

* See refs. [2, 4, 6–8].

†‡‖ Values in any vertical column may be interchanged, but those given here are considered to be most likely.

§ Angelic moiety —COCMe=CHMe.
1′ 2′2″ 3′4′

Although the absolute stereochemistry of diterpenoid **2** is not directly proved, biogenetic reasons based on the presence in the same plant of compounds such as gummiferolic (**1**) and *ent*-kaur-16-en-19-oic acids, point toward an identical absolute configuration for margotianin, which must be methyl *ent*-7α-angeloxy-15α-acetoxy-atis-16-en-19-oate (**2**).

It is important to note that margotianin has a natural methyl ester group, and, as far as we know, the extraction and isolation procedures exclude completely the possibility of this arising as an artefact.

EXPERIMENTAL

Mps were determined in a Kofler apparatus and are uncorr. ^1H NMR and ^{13}C NMR spectra were measured at 100 and 25.2 MHz, respectively, in $CDCl_3$ soln with TMS as internal standard. Assignments of ^{13}C chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. Elemental analyses were carried out in this laboratory with the help of an automatic analyser. Plant materials were collected in September 1977 and June 1978 near Batres (Madrid), and voucher specimens (No. 86747) were deposited in the Herbarium of the Faculty of Pharmacy (Madrid 'Complutense' University).

Extraction and isolation of margotianin (**2**). The extraction of the roots of *M. gummifera* (750 g) was carried out as previously described [1]. The less polar chromatographic fractions obtained before the elution of *ent*-kaur-16-en-19-oic and gummi-

ferolic (**1**) acids, were rechromatographed on a Si gel column (800 g) (eluent: petrol–EtOAc 49:1) yielding 380 mg of crude margotianin (**2**) which after PLC (Si gel plates, petrol–EtOAc 24:1) gave 236 mg of pure **2** (0.031% on dry roots).

Dried and finely powdered *M. gummifera* aerial parts (400 g) were treated as described above, yielding *ent*-kaur-16-en-19-oic acid (140 mg), gummiferolic acid (**1**, 5 g) and margotianin (**2**, 96 mg after purification, 0.024% on dry plant material).

Margotianin (**2**). Mp 142–145° (MeOH), $[\alpha]_D^{20°}$ −80.5° (*c* 0.67, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3080, 1650, 920 (exocyclic methylene), 1720, 1695, 1265, 1180, 1160 (angelate, acetate and axial C-19 methyl ester), 3000, 2950, 2865, 1465, 1445, 1385, 1375, 1370, 1320, 1070, 1045, 990, 965, 880, 855, 840, 810. UV λ_{max}^{EtOH} nm (log ε): 218 (3.93). ^{1}H NMR: δ 5.93 (1H, *qq*, J_{vic} = 7 Hz, $J_{allylic}$ = 1 Hz, H-3 angelate), 5.29 (1H, *br s*, $W_{\frac{1}{2}}$ = 3 Hz, H-15), 4.99 (1H, *t*, *J* = 3 Hz, equatorial H-7), 4.93 and 4.84 (1H each, *m*, $W_{\frac{1}{2}}$ = 4 Hz, 2H-17), 3.62 (3H, *s*, —COOMe), 1.96 (3H, *dq*, J_{vic} = 7 Hz, $J_{allylic}$ = 1 Hz, 3H-4 angelate), 1.92 (3H, *s*, —OAc), 1.90 (3H, *q*, $J_{allylic}$ = 1 Hz, 3H-2′ angelate), C-Me singlets at 1.08 (3H-18) and 0.84 (3H-20). ^{13}C NMR: see Table 1. MS (70 eV, direct inlet) *m/e* (rel. int.): 472 (M^+ 1.7), 412 (9), 372 (16), 357 (5), 354 (3), 330 (17), 313 (19), 312 (23), 253 (100), 252 (16), 171 (26), 145 (12), 105 (14), 101 (5), 91 (17). (Found: C, 70.98; H, 8.73. $C_{28}H_{40}O_6$ requires: C, 71.16; H, 8.53%).

Alkaline hydrolysis of **2** *to yield methyl* ent-7α,15α-*dihydroxy-atis*-16-*en*-19-*oate* (**4**). A soln of compound **2** (150 mg) in 2.5 N ethanolic KOH was refluxed for 24 hr. The soln was then extracted with $CHCl_3$ and the $CHCl_3$ extract was dried, filtered and concd *in vacuo* to leave a residue (100 mg) of pure **4**, mp 147–149° (Et_2O–pentane), $[\alpha]_D^{20°}$ +6° (*c* 0.28, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3260 (OH), 3080, 1660, 900 (exocyclic methylene), 1725, 1195, 1160 (axial C-19 methyl ester). ^{1}H NMR: δ 5.01 and 4.95 (1H each, *q*, *J* = 1.5 Hz, 2H-17), 4.01 (1H, *br s*, $W_{\frac{1}{2}}$ = 3 Hz, H-15), 3.74 (1H, *t*, *J* = 3 Hz, H-7), 3.63 (3H, *s*, —COOMe), C-Me singlets at 1.16 (3H-18) and 0.82 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 348 (M^+ 2), 330 (50), 315 (15), 312 (30), 302 (36), 271 (48), 270 (60), 255 (60), 173 (45), 162 (55), 123 (45), 121 (60), 109 (100). $C_{21}H_{32}O_4$ MW 348.

Methyl ent-7α,15α-*diacetoxy-atis*-16-*en*-19-*oate* (**5**). Treatment of compound **4** (30 mg) with Ac_2O–Py in the usual manner gave **5** (30 mg), mp 148–152° (MeOH), $[\alpha]_D^{20°}$ −14° (*c* 0.29, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3080, 1650, 920 (exocyclic methylene), 1720, 1270, 1185, 1160 (acetates and axial C-19 methyl ester). ^{1}H NMR: δ 5.32 (1H, *br s*, $W_{\frac{1}{2}}$ = 2 Hz, H-15), 4.97 and 4.92 (1H each, *q*, *J* = 1.5 Hz, 2H-17), 4.78 (1H, *t*, *J* = 3 Hz, equatorial H-7), 3.70 (3H, *s*, —COOMe), 2.13 and 2.04 (3H each, two —OAc), C-Me singlets at 1.10 (3H-18) and 0.85 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 432 (M^+ 0.5), 372 (55), 357 (17), 330 (78), 313 (50), 312 (82), 297 (28), 270 (35), 253 (100), 252 (55), 237 (50), 107 (46), 91 (51), 79 (39). $C_{25}H_{36}O_6$ MW 432.

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