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DITERPENES AND UMBELLIFERONE DERIVATIVES FROM *HAPLOPAPPUS DESERTICOLA*

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Key Word Index—*Haplopappus desertícola*, *H anthylloides*, Compositae, diterpenes, labdane derivatives, *nor*-labdane, umbelliferone derivatives, dimeric umbelliferone derivative

Abstract—The aerial parts of *Haplopappus desertícola* afforded three *ent*-labdane and one labdane derivative as well as a *nor-ent*-labdane Furthermore, in addition to three known umbelliferone derivatives, a dimeric coumarin was isolated The structures were elucidated by high field ^{1}H NMR spectroscopy

INTRODUCTION

The large genus *Haplopappus* (Compositae, tribe Astereae, subtribe Solidagininae) is distributed over North and South America So far the investigated species mainly gave clerodanes [1–4] but also some labdanes [3], numerous flavanoids [6–9], some umbelliferone derivatives [10, 11], acetylenic compounds [12] and some more widespread compounds We have now studied two Chilean species, *H desertícola* and *H anthylloides*. The results are discussed in this paper

RESULTS AND DISCUSSION

The aerial parts of *H desertícola* Phil afforded germacrene D, the diterpenes **1**, **1a**, **2** and **3** (isolated as their methyl esters), the *nor*-labdane **4**, the umbelliferone derivatives **5** [13], **6** [14] and **7** [15] as well as the dimeric coumarin **8**

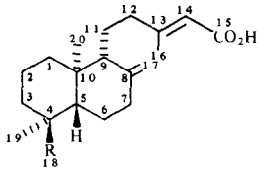
The ^{1}H NMR spectra of **1** and **1a** (Table 1) indicated that we were dealing with diterpenes which must be closely related to agathic acid [16, 17] However, the carbomethoxy group at C-4 was equatorial as could be established by the observed NOE's Thus irradiation of H-20 gave clear effects with H-19 (9%) and H-6 axial (5%). Similarly H-19 gave NOE's with H-20 (10%) and H-6 axial (5%) Furthermore, the optical rotation was opposite, indicating the presence of an *ent*-labdane The ^{13}C NMR data (Experimental) also supported the structure All signals could be assigned by 2D-techniques The relative position of the free acid group in compound **1** followed from the chemical shifts of H-14 and the methoxy group

The ^{1}H NMR spectrum of **2a** (Table 1) showed that it was the corresponding 18-hydroxy derivative. The equatorial orientation of the hydroxy methyl group followed from the chemical shifts of H-18 and H-18′ Also the absence of a *W*-coupling supported this assumption. The enantiomer is copaiferolic acid [18]

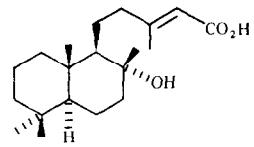
The ^{1}H NMR spectrum of **3a** (Table 1) was similar to those of methyl 8-hydroxy-*ent*-labd-13-*en*-15-oate [19] However, the optical rotation was again opposite, indicating that compound **3** was a normal labdane. The surprising co-occurrence of agathic acid and an *ent*-labdane was observed previously [17]

The ^{1}H NMR spectrum of **4** (Table 1) as well as the molecular formula ($C_{19}H_{30}O_3$) indicated the presence of a *nor*-labdane Most data were similar to those of **1a** except those of the side chain The singlet at δ2.11 and McLafferty fragmentation [M − Me_2CO] indicated the nature of the degraded side chain Accordingly, the ^{1}H NMR signals were close to those of the corresponding compound where the carbomethoxy group was replaced by a methyl group [20] We have named compound **4** methyl haplodesertoate

The structure of **8** could not be deduced directly from the ^{1}H NMR spectrum (Table 2), especially as the mass spectrum gave $C_{14}H_{14}O_3$ as highest fragment, which would be in agreement with compound **5** As, however, the ^{1}H NMR data clearly indicated the absence of the coumarin double bond, a dimer of **5** was proposed This was supported by the ^{13}C NMR spectrum (Table 2) Though only 14 signals were visible, two doublets at δ40 3 and 36 9 required saturated carbons with only one hydrogen each In the ^{1}H NMR spectrum these protons gave two triplet-like signals, which required an AA′,BB′-system It collapsed to a singlet in deuteriobenzene Accordingly, the presence of a dimer of **5** was very likely As photodimerization of coumarin led to four different cyclobutane derivatives [21], the stereochemistry of the dimer had to be elucidated First the question could be solved whether the dimer was a *meso* form or a racemate In the ^{1}H NMR spectrum, after addition of chiral shift reagent, the signals of the aromatic protons were split, indicating the presence of a racemic form However, still a head-to-head *syn* dimer or a head-to-tail *anti* dimer could be present though, from the shape of the ^{1}H NMR signals, an *anti*-arrangement was very likely A clear NOE between H-5 and H-4 established this assumption As UV irradiation of coumarin gives a mixture of isomers with the head-to-head *syn* dimer as by far the main product



1 R = CO_2Me
2 R = CH_2OH



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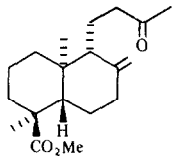

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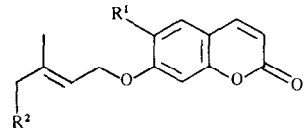
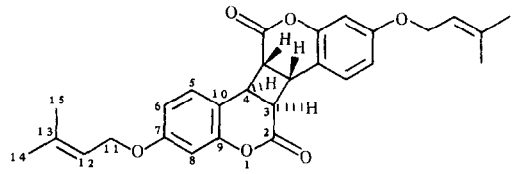


	5	6	7
R^1	H	OMe	OMe
R^2	H	H	prenyl



8

1a–3a are the methylesters

Table 1 ^{1}H NMR spectral data for **1**, **1a**, **2a**, **3a** and **4** ($CDCl_3$, 400 MHz, δ-values)

H	1	1a*	2a	3a†	4
5	1 66 *br d*	1 67 *br d*	1 63 *m*	0 92 *dd*	1 65 *br d*
7	2 24 *m*	2 33 *ddd*	2 36 *ddd*	1 86 *dt*	2 33 *ddd*
7′	2 02 *m*	2 01 *br dt*	1 96 *m*	1 45 *m*	2 00 *br dt*
9	1 93 *dd*	1 93 *dd*	‡	‡	1 94 *dd*
12	2 22 *m*	2 29 *ddd*	2 27 *br dt*	2 29 *br ddd*	2 58 *ddd*
12′	1 98 *m*	1 95 *m*	1 96 *m*	2 18 *br ddd*	2 32 *m*
14	5 67 *tq*	5 65 *tq*	5 63 *tq*	5 68 *tq*	—
16	2 17 *d*	2 16 *d*	2 14 *d*	2 16 *d*	2 11 *s*
17	4 85 *ddd*	4 85 *ddd*	4 83 *ddd*	1 14 *s*	4 83 *ddd*
17′	4 51 *ddd*	4 50 *ddd*	4 48 *ddd*		4 45 *ddd*
18	—	—	3.40 *d* 3 08 *d*	0 86 *s*	—
19	1 13 *s*	1 13 *s*	0 73 *s*	0 78 *s*	1 14 *s*
20	0 71 *s*	0 70 *s*	0 69 *s*	0 77 *s*	0 69 *s*
OMe	—	3 69 *s*	3 68 *s*	3 68 *s*	—
	3 66 *s*	3 66 *s*	—	—	3.65 *s*

*H-6 1 76 *br d*, H-6′ 1 43 *dq*, H-11 1 52 *m*, H-11′ 1 21 *dddd*.

†H-6 1 25 *dq*, H-6′ 1.63 *m*

‡Obscured.

J [Hz]. 5,6 = 12; 6,7 = 4, 6,7′ = 3; 6′,7 = 7,7′ = 13, 6′,7′ = 4 5, 9,11 = 12, 9,11′ = 2.5, 7,17 = 9,17 = 17,17′ ~ 1, 11,12 = 12,12′ = 13, 11′,12 = 4, 12,14 = 14,16 = 1 (compound **1a** 6,6′ = 13)

Table 2 ^{1}H NMR spectral data for **8** ($CDCl_3$, δ-values, 400 or 100 6 MHz respectively)

H	^{1}H NMR	+ Shift reagent	C_6D_6	C	^{13}C NMR	C	
3	4 15 *br t*	4 34 *br s*	3 31 *s*	2	164 3	9	151 3
4	4 25 *br t*	4 36 *br s*		3	40 3	10	108 6
5	7 02 *d*	7 22, 7 20 *d*	7 06 *d*	4	36 9	11	65 0
6	6 67 *dd*	6 74, 6 73 *dd*	6 61 *dd*	5	129 3	12	118 9
8	6 20 *d*	6 29, 6 28 *d*	6 29 *d*	6	103 0	13	138 6
11	4 39 *br d*	4 42 *br d*	4 05 *br dd* 4 00 *br dd*	7	159 7	14	25 8
12	5 40 *tqq*	5 42 *br t*	5 32 *tqq*	8	112 2	15	18 2
14	1 78 *br s*	1 78 *br s*	1 53 *br s*				
15	1 70 *br s*	1 71 *br s*	1 35 *br s*				

J[Hz] 3,4 = 3,4′ = 3′ 4 = 3′,4′ ~9, 5,6 = 8 5, 5,8 = 2 5, 11 12 = 7, 11,14 – 11,15 = 12,14 = 12 15 ~ 1 (in C_6D_6 11,11′ = 17)

[21], compound **8** is most likely already formed in the living plant

The aerial parts of *H anthylloides* Meyen et Walp only gave *p*-hydroxyacetophenone The chemistry of *H. desertıcola* differs from that of most of the species studied so far, by the absence of clerodanes, while the umbelliferone derivatives are of the same type as in other species

EXPERIMENTAL

The air-dried aerial parts (200 g) of *H desertıcola* (collected in Dez 1988 in Chile, Region Metropolitana, voucher AH2, deposited in the Herbarium of the University of Chile Santiago) was extracted with MeOH–Et_2O–petrol (1 1 1) The defatted extract was first separated by CC and further by TLC and HPLC as reported previously [22] The CC fractions were combined to four The first fraction gave 30 mg germacrene D, the second one by HPLC (MeOH–H_2O, 9 1, always RP 8, flow rate 3 ml/min) 20 mg **1** (R_t 4 1 min) and a mixture (R_t 3 2 min) which gave by TLC (Et_2O–petrol, 1 3) 2 mg **1a** (R_f 0 70) and 2 mg **4** (R_f 0 50) The third CC fraction contained 1 5 g **1** The last one was separated by Sephadex LH 20 (CH_2Cl_2–petrol MeOH, 4 7 1) into two crude fractions (4/1 and 4/2) HPLC (MeOH–H_2O, 17 3) of fraction 4/1 gave 150 mg **6** (R_t 2 3 min) 1 g **5** (R_t 3 3 min) and a mixture (R_t 5 9 min) which gave by TLC (Et_2O–petrol, 3 1, two developments) 5 mg **7** (R_f 0 68) and 30 mg **8** (R_f 0 50) Fraction 4/2 showed no methoxy signal in the ^{1}H NMR spectrum After addition of CH_2N_2 HPLC (MeOH H_2O, 17 3) gave 150 mg **2a** (R_t 7 7 min) and 50 mg **3a** (R_t 9 3 min) The aerial parts (490 g) of *H anthylloides* (voucher Niemeyer 8809) only gave 5 mg *p*-hydroxyacetophenone and fatty acids Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material

Methyl-ent-4-epi-*agath*-18-*oate* **1** Colourless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1} 3500–2700, 1690, 1640 (C=CCO_2H), 1725 (CO_2R), MS *m/z* (rel int) 348 230 [M]$^+$ (1) (calc for $C_{21}H_{32}O_4$ 348 230), 333 [M – Me]$^+$ (4), 330 [M – H_2O]$^+$ (2), 299 [330 – OMe]$^+$ (3), 271 [299 – CO]$^+$ (12), 121 [C_9H_{13}]$^+$ (100)

Dimethyl-ent-4-epi-*agathoate* **1a** Colourless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1} 1710, 1645 (C=CCO_2R), 1725 (CO_2R), MS *m/z* (rel int) 362 246 [M]$^+$ (1) (calc for $C_{22}H_{34}O_4$ 362 246), 331 [M – OMe]$^+$ (8), 303 [331 – CO]$^+$ (11), 288 [303 – Me]$^+$ (8), 121 [C_9H_{13}]$^+$ (100), ^{13}C NMR ($CDCl_3$, C-1–C-20) δ 36 9, 18 4, 37 9, 47 6, 55 9, 21 2, 37 7, 147 4, 49 7, 38 9, 26 7, 39 5, 160 8, 114 9, 167 2, 18 8, 106 9, 179 1, 16 5, 14 7, OMe 51 8, 50 7, $[\alpha]_D^{24}$ –35 4 ($CHCl_3$, *c* 7 95)

Methyl-ent-*copaiferolate* **2a** Colourless oil IR $\nu_{max}^{CCl_4}$ cm^{-1} 3650 (OH), 1715, 1645 (C=CCO_2R), MS *m/z* (rel int) 334 251 [M]$^+$ (0 5) (calc for $C_{21}H_{34}O_3$ 334 251), 319 [M – Me]$^+$ (1 8), 304 [M – CH_2O]$^+$ (4), 303 [M – OMe]$^+$ (3), 302 [M – MeOH]$^+$ (2), 274 [302 – CO]$^+$ (1 6), 243 [274 – CH_2OH]$^+$ (4), 55 (100)

Labd-8α-hydroxy-13E-en-15-oic acid **3** Isolatd as its methyl ester **3a**, colourless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1} 3620 (OH), 1720, 1650 (C=CCO_2R), MS *m/z* (rel int) 336 266 [M]$^+$ (5) (calc for $C_{21}H_{36}O_3$ 336 266), 318 [M – H_2O]$^+$ (4), 304 [M – MeOH]$^+$ (22), 114 (100), $[\alpha]_D^{24}$ +14 ($CHCl_3$, *c* 0 13)

Methyl haplodesertoate **4** Colourless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1} 1720 (CO_2R, CO), MS *m/z* (rel int) 306 220 [M]$^+$ (28) (calc for $C_{19}H_{30}O_3$ 306 220) 288 [M – H_2O]$^+$ (50), 273 [288 – Me]$^+$ (14), 248 [M – Me_2CO, McLafferty]$^+$ (57) 246 [M – HCO_2Me]$^+$ (50), 121 [C_9H_{13}]$^+$ (100)

Dimeric umbelliferone-3,3-dimethylallyl ether (**8**) Colourless crystals, mp 100°, IR $\nu_{max}^{CCl_4}$ cm^{-1} 1760 (lactone), 1620 (aromate), MS *m/z* (rel int) 230 094 [M/2]$^+$ (8) (calc for $C_{14}H_{14}O_3$ 230 094), 162 [230 – isoprene]$^+$ (98), 134 [162 – CO]$^+$ (27) 69 [Me_2C=$CHCH_2$]$^+$ (100)

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(−)-SALZOL, AN ISOPIMARANE DITERPENE, AND A CHALCONE FROM *HYPTIS SALZMANII*

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Key Word Index—*Hyptis salzmanii*, Labiatae, flavanone, lignan, 4,2′,6′-trihydroxy-4′-methoxychalcone, isopimarane diterpene, structure elucidation, (−)-salzol

Abstract—From the leaves of *Hyptis salzmanii* a new isopimarane diterpene, named (−)-salzol, and the new 4,2′,6′-trihydroxy-4′-methoxychalcone were isolated, together with three known lignans, i e. (+)-sesamin, (−)-cubebins, (−)-hinokinin, three known flavanones, i e. (−)-isosakuranetin, (±)-sakuranetin, (+)-naringenin-7,4′-dimethylether, and *p*-methoxycinnamic acid. The structure of 20-benzoyloxy-6β,7β,8β,9α-tetrahydroxyisopimar-15-ene, assigned to (−)-salzol, and the structure of the chalcone were determined on the basis of chemical and spectral data

INTRODUCTION

From plants of *Hyptis* genus several cytotoxic agents have been isolated, i e 4′-demethyldesoxypodophyllotoxin, 14-methoxytaxodione, desoxypodophyllotoxin [1, 2] Continuing our studies on biologically active Brazilian plants, we have investigated the methanolic extract of the leaves of *Hyptis salzmanii* Benth. (Labiatae), that showed antimicrobial activity against *S. aureus*, *B subtilis*, *M smegmatis* and *C. albicans* [3] Fractionation of this extract gave several already known compounds together with a new polyhydroxylated isopimarane diterpene named (−)-salzol (**1**) and a new chalcone (**2**)

RESULTS AND DISCUSSION

(−)-Salzol (**1**) was isolated as white needles, mp 196–197°, $[\alpha]_D$ −10.5° ($CHCl_3$) The UV, ^1H (Table 1) and ^{13}C NMR (Table 2) spectra of **1** indicated the presence of a benzoyl residue in the molecule The hydrolysis of salzol under basic conditions gave in fact benzoic acid and the alcohol **1a** ($[M]^+$ at *m/z* 354) The ^{13}C NMR spectrum of **1a** (100 MHz, acetone-d_6, Table 2), showed the resonances of 20 carbon atoms, two of which belong to a vinyl group The remaining signals were assigned to three tertiary methyl groups, five oxygenated aliphatic carbons (1 primary, 2 secondary, 2 tertiary), six methylenes, one methine, and three quaternary aliphatic carbons ^{13}C NMR and mass spectral data indicated the formula $C_{20}H_{34}O_5$ for **1a** and suggested that the compound was a pentahydroxylated diterpene with an isopimarane-type skeleton [4] HETCOR and long-range HETCOR experiments carried on **1** (Table 3) [5, 6], confirmed the supposed structure, and allowed us to locate four hydroxyl groups at the C-6, C-7, C-8 and C-9 positions. The primary alcoholic function was assigned to the C-20 position taking into account the altered chemical shift values of H_2-1, H_2-11 and H_2-20 protons in the ^1H NMR spectrum of **1a** in comparison with those of **1**, and also confirmed by NOE experiments By treatment of **1** with pyridine and acetic anhydride only the monoacetyl derivative **1b** was obtained. The difficulty in obtaining the expected diacetyl derivative was explained by the presence of an intramolecular hydrogen bond between the