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ENT-LABDANE DITERPENOIDS, TREMETONE AND CHROMENE DERIVATIVES AND FLAVONOIDS FROM *OPHRYOSPORUS HEPTANTHUS*

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Key Word Index—*Ophryosporus heptanthus;* Compositae; ent-labdane diterpenoids; tremetone derivatives; chromene derivatives; flavonoids.

Abstract-From aerial parts of *Ophryosporus heptanthus,* one known and three new ent-labdane diterpenoids, four known tremetone and chromene derivatives and two known flavonoids were isolated. Their structures were established by spectroscopic methods.

INTRODUCTION

Earlier studies of the terpenoid chemistry of the genus of *Ophryosporus,* family Compositae, tribe Eupatorieae, subtribe Critoniniae, yielded several prenylated hydroxyacetophenones, ent-labdane derivatives, tremetone derivatives and a cinnamyl ester from 0. *angustifolius* [1], 0. *chilca* [2] and 0. *peruuianus* [2]. In the present report, we describe the isolation from 0. *heptanthus* of one known and three new ent-labdane diterpenoids, four known tremetone and chromene derivatives and two known flavonoids, the 7-monomethyl and 7,4′-dimethyl ether of apigenin.

RESULTS AND DISCUSSION

A dichloromethane extract of ground-leaves of *Ophryosporus heptanthus* afforded compounds **1-10.** Compound 1 was previously reported from another species of the family Compositae, namely, *Corymbium villosum* [3]. Compound 5 was reported from 'White snakeroot'–*Eupatorium urticaefolium* [4], and 6 was reported from a member of *Ophryosporus, 0. angustifolius* [1]. Compounds 7 and 8 were previously reported from *Ageratina aromatica* [5], while 9 and 10 are widespread flavonoids.

Compound **1** was identified as a known natural product by comparing its ^{1}H NMR and MS data with those previously reported [3]. Our observation that 1 was difficult to acetylate, as previously reported [3], was useful for the structure elucidation of the new diterpenoids reported here. An optical rotation result was too small to be reliable, therefore, an ent-labdane skeleton was assigned for 1 based on the ^{1}H NMR, MS and the acetylation studies, which were in accord with those reported [3]. Moreover, an ent-labdane derivative, the deacetyl of 2 was isolated by Zdero, Bohlmann and coworkers [6] and the two compounds exhibited very similar NMR patterns. In the following discussion, most structural features of 2-4 are based on comparison of spectral data with those for 1 and the deacetyl of 2 and with those for each other.

The ^{1}H NMR data for 2 indicated that it also had an ent-labdane-type structure (Table 1), and signals at 64.00 (1H, *ddd, J* = 3, 4, 12 *Hz)* and 63.45 (1H, br *d, J* = 2 Hz) further suggested that 2 had two secondary hydroxyl groups. A 500 MHz 2D-COSY experiment confirmed that the two protons on the two carbon atoms bearing the hydroxyl groups exhibited coupling and therefore were on adjacent carbon atoms. The coupling patterns established that the two hydroxyl groups were at C-2 and C-3. Moreover, a B-orientation of both hydroxyl groups in *2* could be assigned by inspection of Dreiding models of an ent-labdane-type skeleton as found for 1. The CIMS of 2 exhibited a molecular ion at *m/z 365* $[M+H]^+$ *(26%) as* expected for a molecular formula of $C_{22}H_{36}O_4$. Fragments for 2 at *m/z 305* $[M-HOAc]^+$ and 287 $[305-H_2O]^+$ were in accord with fragmentations observed for **1** [3].

The ^{1}H NMR data for 3 (Table 1) indicated that it had a structure similar to **1,** but differed in two respects, namely, by not exhibiting a signal for an acetyl group, thus indicating a primary hydroxyl group at C-15; moreover, a signal at 63.83 for a secondary hydroxyl group could only be assigned to C-2 based on the coupling pattern, which while appearing as *tt* is assigned as *dddd, J* = 4, 4, 11, 11 Hz. In support of a C-2 hydroxyl group was the observation that signals for the two C-4 germinal methyl groups overlapped, a phenomenon typical for this type of compound when there is not a C-3 hydroxyl group. Acetylation of 3 afforded a diacetate **(3a)** which further supported the presence of two hydroxyl groups in 3, and also indicated that the secondary hydroxyl group was not at C-3; following our's and other's [3] observations (as noted above) that a C-3 hydroxyl group in this type of compound is difficult to acetylate. The EIMS data (see Experimental) indicated ready loss of water and the sidechain; this supported the structure assignment for 3. A 2β-hydroxyl group was assigned on the basis of the coupling constants with respect to inspection of Dreiding models.

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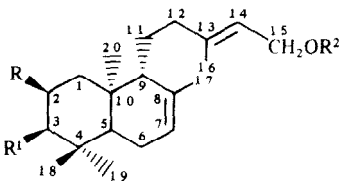
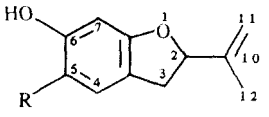
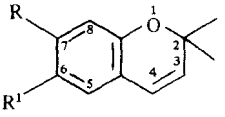


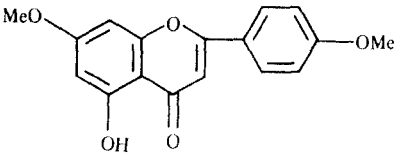
	R	R^1	R^2
1	H	OH	Ac
2	OH	OH	Ac
3	OH	H	H
3a	OAc	H	Ac
4	OH	H	Ac



5 R = OMe
6 R = Ac



	R	R^1
7	OMe	OMe
8	OH	Ac



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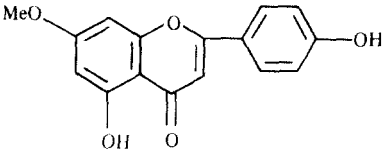

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Table 1. ^{1}H NMR data of compounds **2–4** (360 MHz, $CDCl_3$, TMS)*

H	1	2	3	4
2		4.00 ddd (3, 4, 12)	3.83 dddd (4.4, 11, 11)	3.78
3	3.40 *br s*	3.45 ***hr d*** (3)		
7	5.37 ***br s***	5.42	5.39	5.35
14	5.28 *br t* (7)	5.33	5.40	5.28
15	4.53 ***m***	4.58	4.13 ***hr d*** (7)	4.53 ***m***
16†	1.66 ***hr s***	1.69	1.70	1.64
17†	1.68 *hr s*	1.72	1.70	1.66
18	0.91 *s*	1.00	0.92	0.86
19	0.86 *s*	0.92	0.92	0.86
20	0.74 *s*	0.83	0.76	0.74
OAc	2.02 *s*	2.06		2.00 *s*

*Coupling patterns and coupling constants (*J* in Hz in parentheses) are not repeated if they are identical with the preceding column. Integration is one proton for signals for H-2, 3, 7 and 14, two protons for H-15 and three protons for the H-16, 17, 18, 19, 20 and OAc.

†Assignments for H-16 and H-17 are interchangeable within the column.

Compound 4 differed from 3 in that the primary hydroxyl group in 3 was acetylated in 4 (signal at 62.00, **3H,** s, for an acetyl group). Moreover, the chemical shift of the signals for the two C-15 protons (**δ4.53, 2H,** m), when compared with the signals for the two C-15 protons in 3 (64.13, **2H, *br d),*** indicated a C-15 acetyl function. The CIMS of 4 with a peak at *m/z* 349 $[M + H]^+$ indicated a formula of $C_{22}H_{36}O_3$, and the ^{13}C NMR data supported two oxygenated carbon atoms (661.3 and 65.3) and an acetyl group (*δ* 171.2 and 21.9). The remaining ^{13}C NMR signals as well as MS fragments supported the structure assignment. Again, the *ent*-labdane skeleton was assigned on the basis of comparison of the data for **1** and its related compounds [3, 6] with the data for 4. A C-2*β*-hydroxyl

group was assigned by inspection of Dreiding models with respect to coupling constants. Finally, the structure of 4 was confirmed when acetylation of 4 gave the diacetate **3a.**

Compound 5 (hydroxytremetone) was isolated as a mixture of 5 and 7 (in a ratio *ca* 2:1). We were able to subtract signals for 7 from both the ^{1}H NMR and MS spectra of the mixture because a pure sample of 7 was isolated in the study. Since earlier studies confirmed its structure by synthesis [4, 7], only some spectral data are provided here (Experimental) for comparison.

EXPERIMENTAL

Ophryosporus heptanthus (Wedd.) H. Rob. & King was collected in June 1986 by M. Warnock ca 3-5 km S of Tarata, Peru. A voucher specimen (Warnock 5077) is on deposit in the Plant Resources Center at the University of Texas at Austin.

Dried leaves and flowerheads (965 g) were extracted with CH_2Cl_2 (9 1, 10 min × 2). The combined extracts were evapd under red. pres. to yield a residue which was then dissolved in Me_2CO and the soln stored in a refrigerator overnight. The usual work-up procedure [8] yielded 64 g of gummy material. Part of this concentrate (27 g) was applied to a silica gel column which was then eluted with hexane with increasing amounts of EtOAc. Concentrates of fractions from the column were further purified over Sephadex LH-20 columns (eluting with a 7:4: 1 mixture of cyclohexane–CH_2Cl_2–MeOH and/or prep. TLC (silica gel developed with hexane–EtOAc, 2:1). Compounds 1 (130 mg), 2 (230 mg), 3 (240 mg), 4 (8 mg), **5** and 7 (3 mg, ca 2: 1 mixture based on ^{1}H NMR), 6 (22 mg), 7 (100 mg), 8 (150 mg), 9 (6 mg) and 10 (8 mg) were obtained. Compounds 1 and **6–8** were characterized by comparison of their spectroscopic properties with literature values [1, 3, 5], while 9 and **10** were identified following standard procedures [9].

Attempted *acetylation of* **1.** Compound 1 (62 mg) was treated with Ac_2O–pyridine in the usual manner for 5 hr. Both TLC and ^{1}H NMR showed that no acetylation had occurred.

*2β,3β-Dihydroxy-15-acetoxy-*ent*-labda-7,13E-diene* (2). Colourless gum. CIMS, methane, m/z (rel. int.): 365 $[M+H]^+$ $(C_{22}H_{36}O_4)$ (26), 305 $[365-HOAc]^+$ (62), 287 $[305-H_2O]^+$ (100).

*2β,15-Dihydroxy-*ent*-labda-7,13E-diene* (3). Colourless gum. EIMS (probe) 70 eV, m/z (rel. int.): 288 $[M-H_2O]^+$ $(C_{20}H_{34}O_2$, 306) (1), 220 $[288-$sidechain$]^+$ (98), 188 $[288-C_6H_{12}O]^+$ (22), 121 $[188-C_5H_7]^+$ (44), 106 $[121-Me]^+$ (48), 91 $[106-Me]^+$ (52), 81 $[C_6H_9]^+$ (100).

Acetylation of 3. Compound 3 (20 mg) was acetylated with Ac_2O–pyridine in the usual manner for 1 hr to afford 21 mg of the diacetate 3a. ^{1}H NMR (200 MHz, CDCI,, TMS) 64.99 (1H, *dddd*, *J* = 4, 4, 12, 12 Hz, H-2), 5.40 (1H, *br s*, H-7), 5.35 (1H, *br t*, *J* ≈ 7 Hz, H-14), 4.59 (2H, *br d*, *J* = 7 Hz, H-15), 1.71 (6H, *br s*, H-16 and 17), 0.97 (3H, s, H-18), 0.92 (3H, *s*, H-19), 0.84 (3H, s, H-20), 2.06 (3H, s, OAc), 2.04 (3H, s, OAc).

*2β-Hydroxy-15-acetoxy-*ent*-labda-7,13E-diene* (4). Colourless gum. CIMS MeOH, *m/z* (rel. int.): 349 $[M+H]^+$ $(C_{22}H_{36}O_3)$ (14), 289 $[M-OAc]^+$ (65), 271 $[289-H_2O]^+$ (100), 220 $[M-HOAc-C_5H_8]^+$ (8). ^{13}C NMR (125 MHz, $CDCl_3$, N and P were Attached Proton Test results) 6: 34.1 (P, C-l), 65.3 (N, C-2), 48.1 (P, C-3), 38.4 (P, C-4), 49.4 (N, C-5), 25.3 (P, C-6), 122.2 (N, C-7), 134.8 (P. C-S), 54.0 (N, C-9), 51.0 (P, C-10), 23.5 (P, C-l 1), 41.6 (P, C-12), 142.4 (P, C-13), 118.4 (N, C-14), 61.3 (P, C-15), 22.7 (N, C-16), 16.5 (N, C-17), 33.0 (N, C-18), 21.0 (N, C-19), 14.3 (N, C-20), 21.9 (N) and 171.2 (P). (Ac).

Acetylation of 4. Compound 4 (17 mg) was acetylated with Ac_2O–pyridine in the usual manner to yield 20mg of 3a as determined by ^{1}H NMR.

Mixture of *5-methoxy-6-hydroxytremetone* (5) and precocene II (7). Colourless gum. EIMS (probe) 70 eV, m/z (rel. int.) (for 5): 206 [M]' $(C_{12}H_{14}O_3)$ (30), 191 [M-Me]+ (16), 173 $(191-H_2O]^+$ (18). 161 [191 -2 x Me]+ (18). (for 7): 220 $[M]^+$ $(C_{13}H_{16}O_3)$ (79), 205 $[220-Me]^+$ (100), 189 $[M-OMe]^+$ (32), 174 $[189-Me]^+$ (16). ^{1}H NMR (360 MHz, $CDCl_3$, TMS) δ 5.18 (1H, *br t*, *J* = 9 Hz, H-2), 3.29 (1H, *br dd*, *J* = 9, 15 Hz, H-3), 2.98 (1H, brdd, *J* = 9, 15 Hz, H-3'), 6.73 (1H, *br s*, H-4), 6.47 (1H, s, H-7), 5.07 (1H, *br s*, H-11), 4.90 (1H, *br s*, H-11'), 1.77 (3H, *br s*, H-12), 3.82 (3H, *s*, OMe).

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