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FLAVONOIDS AND TERPENOIDS OF *CHENOPODIUM GRAVEOLENS**

RACHEL MATA,‡ ANDRÉS NAVARRETE, LAURA ALVAREZ,† ROGELIO PEREDA-MIRANDA,† GUILLERMO DELGADO† and ALFONSO ROMO DE VIVAR†

División de Estudios de Posgrado, Facultad de Química, Ciudad Universitaria, Circuito Escolar, Coyoacán 04510 México, D.F.; †Instituto de Química, Ciudad Universitaria, Circuito Exterior, Coyoacán 04510 México, D.F.

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Key Word Index—*Chenopodium graveolens*; Chenopodiaceae; flavonoids; pinostrobin; pinocembrin; chrysin; sesquiterpenoids; (+)-8α-hydroxyelemol; (+)-8α-acetoxycryptomeridiol; cryptomeridiol.

Abstract—Three flavonoids, four terpenoids and three steroids were isolated from *Chenopodium graveolens*. These included pinostrobin, stigmasterol, stigmast-22-en-3-ol, 3α-sitosteryl-glucoside, geranyl acetate, pinocembrin, chrysin, cryptomeridiol, and two new sesquiterpenes which were characterized by spectral means as (+)-8α-hydroxyelemol and (+)-8α-acetoxycryptomeridiol.

INTRODUCTION

Chenopodium graveolens Willd., known in Mexico as Epazote de zorrillo, is widely used in traditional medicine to improve gastrointestinal upsets and for the treatment of worms [1–3]. Previous chemical work on the genus deals mainly with the ascaridole content of the essential oils [4–9] or with the flavonoids [10–14]. The following compounds have also been reported: betacyanins in *C. botrys* [15] and *C. urbicum* [16], ecdysteroids in *C. album* [17] and *C. bonus-Henricus* [18], triterpenoid glucosides in *C. antihelminticum* [19] and *C. ambrosioides* [20], and polyoxygenated sesquiterpenes in *C. botrys* [21–23].

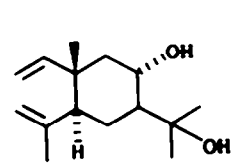
We report herein the identification in *C. graveolens* of ten compounds, two of which are new sesquiterpenoids.

RESULTS AND DISCUSSION

By initial preparative column chromatography over silica gel and subsequent column rechromatography on silica gel impregnated or not with silver nitrate, the chloroform extract of *C. graveolens* afforded the flavonoids pinostrobin, pinocembrin and chrysin, three steroids, as well as the terpenoids geranyl acetate and 1–3.

(+)-8a-Hydroxyelemol, **1**, ($C_{15}H_{26}O_2$), mp 110°, was obtained as colourless needles. The IR spectrum (see Experimental) showed secondary and tertiary hydroxyl group absorptions as well as bands corresponding to olefinic unsaturation. No molecular ion (*m/z* 238) was observed in the electron-impact mass spectrum but fragment ions at M − 15, M − 18, M − 18 − 15 and M − 18 − 18 showed that **1** contains two hydroxyl groups; other important ions were at *m/z* 59 (base peak) and *m/z* 43, corresponding to $[Me_2C{=}OH]^+$ and $[Me_2C{\equiv}O]^+$ fragments, respectively, and which resulted from cleavage adjacent to the hydroxyl function of a 2-isopropanol moiety [24]. The ^{1}H NMR and ^{13}C NMR spectra (Tables 1 and 2) confirmed these assignments and demonstrated the elemene nature of **1** (signals at δ 5.77, 4.92, 4.90 and 4.82 in the ^{1}H NMR spectrum and at δ 149.3, 146.5, 112.3 and 110.7 in the ^{13}C NMR spectrum; the chemical shift observed for both the C-14 and C-15 methyl groups in the ^{13}C NMR spectra were in agreement with the stereochemistry at C-10 and C-5. The location and disposition of the hydroxyl group at C-8 were established by the observed coupling pattern of the signal at δ3.97 (Table 1), which is consistent with the carbinol proton being in a *trans*-diaxial relation with two vicinal protons, as well as in a *cis* relation with a third vicinal proton (it is possible only if the secondary hydroxyl group is placed at C-8 with an α-orientation). It must be mentioned that the ^{1}H NMR spectrum of this compound is very similar to that of botridiol, differing mainly in the coupling pattern of the carbinol proton and in the chemical shift of the isopropyl side chain methyls [22].

(+)-8α-Acetoxycryptomeridiol, **2**, ($C_{17}O_4H_{30}$), mp 70°, exhibited bands at 3691, 3597, 1731 cm^{-1} in its IR spectrum. The chemical ionization mass spectrum revealed important ions at M + 1 − 18, M + 1 − 18 − 18, M + 1 − 18 − 58, M + 1 − 60 − 18, M + 1 − 60 − 18 − 18 and M + 1 − 18 − 58 − 60. The above spectral findings suggested that **2** is the monoacetate of a sequiterpene diol with a eudesmane skeleton [25]. The ^{1}H NMR spectrum (Table 1) displayed above the 2 ppm region three signals for the skeletal methyl groups, very similar to those in the spectrum of cryptomeridiol (**3**), and with both methyl groups of the C-7 side chain exhibiting the same chemical



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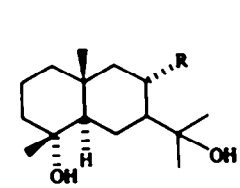

2 R = OAc
3 R = H

*Part 1 in the series "Chemical Studies on Mexican Plants Used in Traditional Medicine". Taken in part from the MS research work of A. Navarrete.

‡To whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data of sesquiterpenes 1-3 (80 MHz, $CDCl_3$, TMS as internal standard)

H	1	2	3
H-1	5.77 *dd* (16, 10)	—	
H-2	4.92 *m*		—
H-2'	4.90 *m*	—	
H-3	4.80 *m*	—	
H-3'	4.65 *br s*		
OH	3.15 *s**	1.90	1.60 *s**
H-8	3.97 *ddd* (6, 10, 10)	5.025 *dd* (6, 10, 10)	—
H-12	1.26 *s*†	1.21 *s*†	1.19 *s*†
H-13	1.31 *s*†	1.21 *s*†	1.19 *s*†
H-14	1.03 *s*†	0.96 *s*†	0.85 *s*†
H-15	1.75 *br s*†	1.10 *s*†	1.09 *s*†
MeCO		2.01 *s*†	

Figures in parentheses are coupling constants in Hz.
*Disappeared after equilibration with D_2O.
†Intensity three protons.

Table 2. ^{13}C NMR spectral data of sesquiterpenes 1 and 2*

C No.	1	2
1	149.3 (*d*)	40.0 (*t*)
2	112.3 (*t*)	22.2 (*t*)
3	110.7 (*t*)	29.6 (*t*)
4	146.5 (*s*)	70.3† (*s*)
5	53.5 (*d*)	52.9 (*d*)
6	29.2 (*t*)	35.1 (*t*)
7	51.5 (*d*)	51.6 (*d*)
8	67.0 (*d*)	70.6 (*d*)
9	40.7 (*t*)	43.0 (*t*)
10	48.4 (*s*)	50.1 (*s*)
11	73.0 (*s*)	70.1 (*s*)†
12	24.75 (*q*)	21.5 (*q*)†
13	28.4 (*q*)	27.9 (*q*)
14	17.5 (*q*)	18.6
15	24.5 (*q*)	21.0 (*q*)
MeCO		169.6 (*s*)
$CH_3\underline{C}O$		19.5 (*q*)

*Run on a 50 MHz instrument in DMSO-d_6 with TMS as internal standard. Signals were assigned by means of off-resonance decoupled spectra by using previously reported model compounds.
†Might be interchangeable.

shift [25]. The chemical shift equivalence of H-12 and H-13, as well as the lack of bands for associated acetoxyl groups in the IR spectrum, ruled out, at first, the placement of the ester moiety on C-6 [22, 25]. As in **1**, the position and stereochemistry of the acetoxyl group on C-8 were deduced from the coupling pattern of the signal at δ 5.03. Structure **2** was also in accord with the ^{13}C NMR spectrum (Table 2).

Cryptomeridiol (**3**) showed similar physical and spectral characteristics to those reported previously [25]. Chrysin showed similar UV and IR spectra to those previously published. Furthermore, the physical constants, as well as the spectral properties of the diacetate and the monomethyl ether, were identical to those described in the literature [26–28]. The flavanones pinostrobin and pinocembrin were identified by comparison of their spectral characteristics and physical data with those reported [29–32]; additional evidence was provided by chemical correlation, since methylation of pinocembrin with ethereal diazomethane afforded pinostrobin.

Although flavonols have previously been detected in several *Chenopodium* species, this is the first time that flavones and flavanones have been described. Whereas *Chenopodium* flavonols are all oxygenated in the B-ring [10–14], these flavones and flavanones lack oxygenated substituents in this ring. By contrast, sesquiterpenes **1** and **2** are both substituted at C-8, while the related species *C. botrys* has many sesquiterpenes bearing substituents at C-6 or C-3 [21, 23].

EXPERIMENTAL

Plant material. Chenopodium graveolens Willd. was obtained from El Mercado de Sonora, Mexico, D.F. Reference specimens have been deposited at the National Herbarium (voucher No. GDL-1158).

Extraction and preliminary fractionation. The dried and shredded aerial parts of the plant (3.1 kg) were macerated (× 3) for 3-day periods with $CHCl_3$ at room temp. The combined $CHCl_3$ extracts were pooled and evaporated under reduced pressure to afford a brownish residue (103.5 g), which was then subjected to CC over silica gel (3 kg) using hexane, hexane–$CHCl_3$ in different proportions, $CHCl_3$, $CHCl_3$–Me_2CO in different proportions, and Me_2CO as eluants, 500 ml fractions being collected.

Isolation of pinostrobin. From fractions 121–146 eluted with hexane–$CHCl_3$ (1:1), a crystalline powder was separated and recrystallized from Et_2O to yield 3 g (0.1% of dry wt), mp = 100° (lit. [29–30] mp 99–100°).

Isolation of stigmasterol, stigmast-22-en-3-ol and geranyl acetate. Fractions 147–214 (6.1 g) from the original column were rechromatographed on silica gel (100 g), starting the elution with hexane and then with hexane–EtOAc (9:1). Fractions 24–29 gave 100 mg (0.003% of dry wt) of stigmasterol, mp 169°, which was characterized by comparison with an authentic sample. Fractions 31–39 yielded 75 mg (0.0025% of dry wt) of stigmast-22-en-3-ol, identical in all respects with an authentic sample. Finally from fractions 90–100 300 mg (0.01% of dry wt) of geranyl acetate was obtained as a colourless oil, which was identical to a standard sample.

Isolation of pinocembrin. From fractions 310–312 (5.45 g) 500 mg of a greenish powder, mp 90° precipitated; 200 mg of this powder was dissolved in MeOH and treated with an excess of CH_2N_2 in Et_2O at room temp. The mixture was left overnight and then evaporated. The crystalline residual material, recrystallized once from Et_2O, afforded 63 mg pinostrobin.

The remaining part of the fraction (4.75 g) was further rechromatographed on silica gel (200 g) using hexane–$CHCl_3$ (1:1), $CHCl_3$ and $CHCl_3$ with increasing amounts of EtOAc as the mobile phase. Fractions 47–59 eluted with $CHCl_3$–EtOAc (19:1) were acetylated with Ac_2O–pyridine (1:1) at room temp. After work-up of the reaction mixture, 500 mg pinocembrin diacetate, mp 105°, was obtained. The total yield of pinocembrin was 0.525 g (0.0175% of dry wt).

Isolation of chrysin. From fractions 313-315 (eluted with $CHCl_3$–Me_2CO, 9:1) of the initial column crystallized 1.5 g (0.05% of dry wt) of chrysin, mp 275–280°, (lit. [26–28] mp

289–290°); diacetate, mp 198° (lit. [26–28] mp 198–201°); monomethyl ether, mp 177–179° (lit. [28] mp 177–180°).

Isolation of (+)-8α-hydroxyelemol (**1**). Fractions 316–350, eluted with $CHCl_3$–Me_2CO (9:1) from the initial column, were filtered over activated charcoal and tonsil (1:1, 100 g). The filtered soln was concentrated to afford a residue (4.34 g), which was then rechromatographed on silica gel (150 g). Elution was started with hexane–EtOAc (9:1) and then continued with increasing amounts of EtOAc. Fractions 45–99 (2 g) eluted with hexane–EtOAc (4:1) were further chromatographed on silica gel (80 g) impregnated with $AgNO_3$ (10%) using $CHCl_3$–EtOAc (19:1) as eluant. Fractions 48–64 afforded 140 mg **1** (0.0036% of dry wt), mp = 110°, after filtration over activated charcoal, and recrystallization from Et_2O. (Calc. for $C_{16}H_{26}O_2$: C, 75.68; H, 11.1. Found: C, 75.40; H, 10.9%.) $[\alpha]_D$ +0.01° (*c* 0.78; MeOH); UV λ_{max}^{MeOH} nm (log ε): 202 (1.7); CIMS *m/z* (rel. int.): 239 [M + 1]$^+$ (4.4), 221 (15), 203 (100), 147 (60), 109 (32), 107 (30); EIMS *m/z* (rel. int.): 223 (5), 220 (5), 205 (14), 202 (15), 162 (10), 157 (15), 107 (50), 79 (58.2), 59 (100), 43 (92), 41 (89); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3605, 3450, 3084, 2973, 1639, 1377, 1168, 1048, 898; ^{1}H NMR (80 MHz, $CDCl_3$): see Table 1.

Isolation of (+)-8α-acetoxycryptomeridiol (**1**) *and (−)-cryptomeridiol* (**3**). Fractions 374–405 (5.51 g), eluted with $CHCl_3$–Me_2CO (9:1) of the initial column, were rechromatographed on silica gel (200 g). Elution was done with $CHCl_3$ and increasing quantities of EtOAc. From fractions 134–153 110 mg (0.0036% of dry wt) of **2**, mp 70°, was obtained; $[\alpha]_D$ +0.10° (*c* 2; $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 203 (1.78); CIMS *m/z* (rel. int.): 299 [M + 1]$^+$ (5), 281 (100), 263 (10), 221 (48.9); EIMS *m/z* (rel. int.): 95.3 (12), 59.2 (40), 43 (100), 41.2 (15); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3691.9, 3567, 3006, 2934, 1731, 1602, 1387, 1166, 910; ^{1}H NMR (80 MHz, $CDCl_3$): see Table 1. Finally, fractions 154–169 eluted with the same solvent polarity afforded 90 mg **3** (0.003% of dry wt), mp 130° (lit. [25] mp 134.5–135.5°).

Isolation of 3β-sitosteryl glucoside. Fractions 414–418 (2.84 g) eluted with $CHCl_3$–Me_2CO (1:1) of the initial column were rechromatographed on silica gel (120 g). Elution was accomplished with EtOAc with increasing amounts of Me_2CO. Fractions 21–35 afforded a crystalline powder, which after recrystallization from Et_2O yielded 110 mg 3β-sitosteryl glucoside (0.0036% of dry wt), which was identical to a reference sample.

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