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NEW SECO-PSEUDOGUAIANOLIDES AND OTHER TERPENOIDS FROM A POPULATION OF *AMBROSIA CONFERTIFLORA*[1,2]

GUILLERMO DELGADO,* MARÍA ISABEL CHÁVEZ, and LAURA ALVAREZ

Instituto de Química de la Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, D.F.

ABSTRACT.—The aerial parts of *Ambrosia confertiflora* afforded, in addition to clovandiol, psilostachyin, psilostachyin B, psilostachyin C, desacetylconfertiflorin, stigmasterol, and β-sitosterol, two new sesquiterpene lactones, 1′-noraltamisin [**6**] and (11*R*)-11,13-dihydropsilostachyin [**7**], whose structures were elucidated by spectroscopic methods and chemical correlations.

Natural products isolated from *Ambrosia* species exhibit a wide variety of potent and interesting biological activities (1). In particular, sesquiterpene lactones isolated from *Ambrosia maritima* (2,3) and *Ambrosia confertiflora* DC. (Compositae) (4) show molluscicidal properties against the intermediate hosts of schistosomiasis. The latter species has been the subject of several studies, because it exhibits considerable infra-specific variation in its sesquiterpene lactones (5), which are used as taxonomic characters at various levels (6,7). Here we report the isolation and chemical characterization of the terpenoid constituents of a Mexican population of *A. confertiflora*.

RESULTS AND DISCUSSION

A $CHCl_3$ extract of the aerial parts of *A. confertiflora* afforded seven terpenoids, shown to be identical with stigmasterol, β-sitosterol, clovandiol [**1**], psilostachyin [**2**], psilostachyin B [**3**], psilostachyin C [**4**], and desacetylconfertiflorin [**5**] by comparison (^{1}H nmr, ms, ir) with authentic samples. The new compounds 1′-noraltamisin [**6**] and (11*R*)-11,13-dihydropsilostachyin [**7**] were also isolated, and their structures were determined as follows.

1′-Noraltamisin [**6**] has the molecular formula $C_{16}H_{22}O_5$ on the basis of elemental analysis and ms. The ir spectrum of **6** showed it to be an α,β-unsaturated-γ-lactone (1766, 1670 cm^{-1}), containing a hydroxyl group (3592 cm^{-1}) and an ester moiety (1728 cm^{-1}). The alcohol is tertiary, inasmuch as **6** was unchanged on attempted acetylation. The ^{1}H-nmr spectrum of **6** (Table 1) displayed two one-proton doublets at

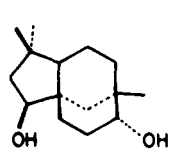
TABLE 1. ^{1}H-nmr Parameters of **6**, **7**, and **13**.[a]

Proton	Compounds			
	6 ($CDCl_3$)	**7** ($CDCl_3$)	**7** (C_6D_6)	**13** ($CDCl_3$)
H-6	4.69 d (9.0)	4.89 d (9.0)	4.57 d (9.0)	4.60 d (9.0)
H-7	3.31 m			
H-13	6.19 d (3.5)	1.25 d (7.0)	0.67 d (7.0)	1.18 d (7.0)
H-13′	5.48 d (3.4)			
H-14	1.72 s	1.09 d (7.0)	0.47 d (7.0)	0.97 d (7.0)
H-15	1.20 s	1.26 s	0.88 s	1.52 s

[a]Chemical shifts were recorded at 80 MHz and are expressed in δ ppm from TMS. Coupling constants are in parentheses (Hz).

[1]Dedicated to Professor Dr. Alfonso Romo de Vivar, Universidad Nacional Autónoma de México, on the occasion of his 30 years of teaching and research.

[2]Contribution No. 876 of Instituto de Química de la UNAM.



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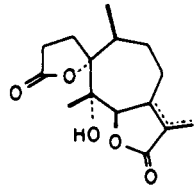
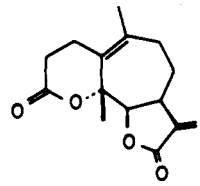


2 $\Delta^{11,13}$
12 $\Delta^{7,11}$



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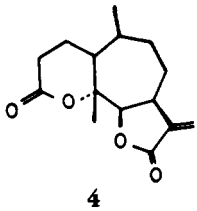

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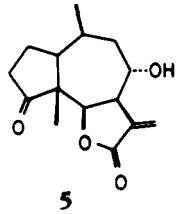

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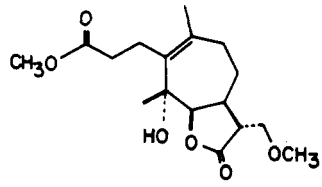

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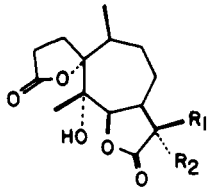
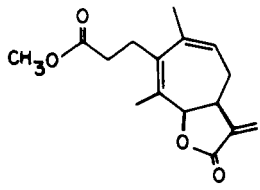


7 R_1=Me, R_2=H
13 R_1=H, R_2=Me



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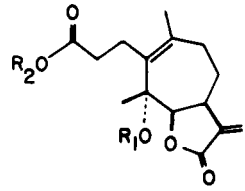

6 R_1=H, R_2=Me
8 R_1=TAC, R_2=Me
9 R_1=H, R_2=Et

δ 6.19 and δ 5.48 (J=3.5 Hz) and a broad one-proton multiplet at δ 3.31 that are characteristic for H-13, H-13′, and H-7, respectively, of α,β-unsaturated-γ-sesquiterpene lactones. The three-proton singlet at δ 3.64 indicated the presence of a methyl ester. Moreover, the high-field singlets at δ 1.72 and δ 1.20 revealed the presence of vinylic and carbinolic methyls, respectively. These data together with the doublet at δ 4.69 (J=9 Hz) are in accord with a hydroxy-seco-pseudoguaianolide containing a *cis*-lactone (7).

The down-field shift of H-6 ($\Delta\delta$=0.11) and H-15 ($\Delta\delta$=0.34) in the trichloroacetylcarbamate (TAC) **8** [obtained in situ upon treatment with trichloroacetyl isocyanate (8)] established that the tertiary hydroxyl is at C-5 and is *cis*, and hence, the tetrasubstituted double bond is at C(1)-C(10). Consequently, the new sesquiterpene possesses the structure **6**, and comparison with the spectroscopic data of altamisin [**9**] (9) supports this proposal, which was confirmed by the following chemical correlations.

Treatment of **3**, also isolated from this species with MeOH and acid, gave the previously described (10) diene **10**. The same oily diene was obtained when 1′-noraltamisin [**6**] was similarly treated.

In addition, the same Michael adduct **11** was obtained when either psilostachyin B [**3**] or 1′-noraltamisin [**6**] reacted with methanolic sodium methoxide (10).

The second new sesquiterpene [**7**] had the composition $C_{15}H_{22}O_5$ (elemental analysis and ms). The ir spectrum displayed hydroxyl (3588 cm^{-1}) and carbonyl (1769 cm^{-1}, broad) absorptions. No absorption was present above 200 nm, and **7** was not acetylated by Ac_2O and pyridine, suggesting that the hydroxyl group is tertiary. The ^{1}H-nmr spectrum of **7** (Table 1) showed a one-proton doublet at δ 4.89 (J=9 Hz) for the lactonic methine, a three-proton singlet at δ 1.26, corresponding to a carbinolic

methyl group, and two three-proton doublets of secondary methyls. These high-field signals, together with the absence of those for vinylic protons, suggest that **7** is a 11,13-dihydro derivative of psilostachyin, in agreement with the molecular formula.

Chemical evidence to support the proposed structure was provided as follows. Prolonged catalytic hydrogenation of psilostachyin [**2**] afforded, in addition to isopsilostachyin [**12**], a dihydroderivative, identical with that obtained from the $NaBH_4$ reduction of **13**, as reported earlier (11). However, the authors did not establish the stereochemistry at C-11, and this product is different from our natural dihydropsilostachyin. Presumably, prolonged catalytic hydrogenation of **2** allowed equilibration of the methyl group at C-11 (from β to α).

This was shown to be so. Thus, catalytic reduction of **2** for a short time afforded, in addition to isopsilostachyin [**12**], the dihydroderivative **7**, identical in all respects with the natural product isolated from *A. confertiflora*. The (11*R*)-configuration of **7** is based on the catalyst approaching from the less encumbered α-side. Treatment of **7** with methanolic K_2CO_3 gave the equilibration product (11*S*)-11,13-dihydropsilostachyin [**13**], identical with that obtained by $NaBH_4$ reduction of **2**. ^{1}H-nmr data of both epimers are included in Table 1 for comparison.

A small amount of a dihydropsilostachyin of unknown stereochemistry at C-11 has previously been isolated from *Ambrosia arborescens* (12). Comparison of their physical and spectroscopic data indicated its identity with (11*S*)-11,13-dihydropsilostachyin.

The chemistry of this population of *A. confertiflora* is similar to other populations previously investigated. If one accepts that there are four major chemotypes differing in the composition of their sesquiterpene lactones, then the population examined belongs to the psilostachyin-type chemotype (6).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Jones apparatus and are uncorrected. Analytical tlc was carried out on Si gel 60F-254 plates (Merck) and silica 70-230 mesh (Merck) was used for column chromatography. Ir spectra were determined on a Perkin-Elmer 283B spectrometer. Nmr spectra ($CDCl_3$; δ ppm/TMS) were recorded on a Varian FT-80 apparatus. Mass spectra were obtained on a Hewlett-Packard 5985-B spectrometer by direct inlet probe at 75 eV.

PLANT MATERIAL.—*A. confertiflora* was collected in September 1984, 12 km north of Zacatecas along Hwy. 45. A voucher specimen (GD-1160) is deposited in the National Herbarium, Instituto de Biología de la Universidad Nacional Autónoma de México.

PLANT EXTRACTION AND PRELIMINARY FRACTIONATION.—Dried and powdered aerial parts of the plant (4.9 kg) were extracted three times with $CHCl_3$. The residue obtained after evaporation of the solvent was defatted at 0° with *n*-hexane. The extract (58 g) was chromatographed directly on Si gel (1.8 kg), the column eluted with *n*-hexane/EtOAc mixtures, and 750-ml fractions collected.

ISOLATION OF TERPENOIDS.—The fractions eluted with *n*-hexane contained waxes and fats. Stigmasterol (2.80×10^{-2}% of dry wt) was the main component in fractions 41-68, eluted with *n*-hexane-EtOAc (9:1). Fractions 60-73 gave β-sitosterol (1.10×10^{-2}% of dry wt). Fractions 112-125, eluted with *n*-hexane-EtOAc (7:3) gave a crystalline powder (0.0122% of dry wt) which was recrystallized from Me_2CO/iPr_2O to give 1′-noraltamisin [**6**], mp 93-95°;

$$[\alpha]^{\lambda}{}_{21^\circ}=\frac{589}{+34.1}\quad\frac{578}{+41.0}\quad\frac{545}{+43.2}\quad\frac{436}{+82.4}\quad\frac{365}{+163.0}\ (c=0.2,\ \text{MeOH});$$

uv λ max nm (MeOH) 205 (ε 13555, C 0.32 mg/ml); ir ($CHCl_3$) cm^{-1} 3592, 1766, 1728, 1670, 1607, 1436, 1379, 1355, 1326, 1280, 1256, 1177, 1048, 986; ^{1}H nmr see Table 1; ms *m/z* (rel. int.) 294 (M^+, 8), 265 (16), 262 (27), 221 (20), 203 (23), 173 (14), 43 (100). Found: C, 65.21; H, 7.54 $C_{16}H_{22}O_5$ requires C, 65.29; H, 7.53. To the ^{1}H-nmr sample of **6** was added two drops of trichloroacetyl isocyanate (TAI), and after 5 min, the spectrum of **8** was recorded. The signals remained unchanged with the exception of $^{15}CH_3$ (δ 1.54, s, 3H) and H-6 (δ 4.80, d, $J=9$ Hz).

From fractions 167-177 eluted with *n*-hexane-EtOAc (1:1), an amorphous solid was separated (0.0460% of dry wt). This precipitate was recrystallized and identified as psilostachyin [**2**], mp 215-218 (11).

Fractions 178-197, eluted with *n*-hexane-EtOAc (2:3) were combined and the residue (28.3 g) rechromatographed on Si gel (500 g), eluting with $CHCl_3/Me_2CO$. Psilostachyin B [**3**] (0.020% of dry wt), mp 113-114° (10) eluted first, and then 0.002% of (11*R*)-11,13-dihydropsilostachyin [**7**], mp 225-226° was obtained; $[\alpha]^{25}D$ −70.0° (*c*=1, MeOH); ir ($CHCl_3$) cm^{-1} 3588, 1769, 1456, 1388, 1352, 1248, 1176, 1109, 1073, 998; ^1H nmr ($CDCl_3$ and C_6D_6 see Table 1); ms *m/z* (rel. int.) 282 (M^+, 8), 264 (12), 206 (20), 191 (18), 181 (16), 151 (15), 139 (29), 138 (45), 125 (100). Found: C, 63.86; H, 7.82. $C_{15}H_{22}O_5$ requires C, 63.81; H, 7.85. From the most polar fractions 10 mg of clovandiol [**1**] was obtained, mp 150-152° (13).

Fractions 198-226 of the original column were combined and the residue (1.51 g) rechromatographed over silica (50 g) using $CHCl_3/Me_2CO$ gradient elution. From the less polar fractions, 350 mg of psilostachyin C [**4**] were obtained, mp 223-225°. The polar fractions afforded 900 mg of desacetylconfertiflorin [**5**], mp 208-210° (15).

DIENE ESTER **10**.—*From psilostachyin B* [**3**].—A solution of **3** (100 mg) in 3 ml MeOH and 0.2 ml H_2SO_4 was allowed to stand for 1.5 h at room temperature. Usual work-up afforded 85 mg of **10** as a pale yellow oil (10).

From 1′-noraltamisin [**6**].—A solution of **6** (116 mg) in 2.5 ml MeOH and 0.15 ml of H_2SO_4 was allowed to stand for 1 h. Usual work-up gave 75 mg residue that was purified by preparative tlc (*n*-hexane-EtOAc, 4:1) to afford 45 mg of **10** as an oil (10); ms *m/z* (rel. int.) 276 (M^+, 40), 258 (24), 226 (17), 203 (23), 202 (30), 189 (77), 187 (32), 173 (25), 161 (32), 157 (39), 145 (30), 143 (45), 137 (46), 115 (40), 107 (51), 105 (52), 91 (100), 77 (57).

METHANOL ADDUCT **11**.—*From psilostachyin B* [**3**].—A solution of **3** (100 mg) in 1 ml MeOH and 0.25 ml of MeONa (prepared from 11 ml MeOH and 100 mg Na) was allowed to stand for 2 h. The solution was neutralized with dilute HCl and worked up as usual to give 80 mg of **11** (10).

From 1′-noraltamisin [**6**].—Similar treatment of **6** (100 mg) afforded 85 mg of crystalline **11**, mp 83-84° (10).

(11S)-11,13-Dihydropsilostachyin [**13**].—Hydrogenation of a solution of **2** (100 mg) with 10% Pd-C (10 mg) for 15 h gave two products, separated by preparative tlc ($CHCl_3$-Me_2CO, 7:3) into a less polar component, mp 194-195° (40 mg), which was identified by direct comparison with isopsilostachyin [**12**] (11). The more polar component **13** was recrystallized from Me_2CO/iPr_2O, mp 222-223° (45 mg). It was identical with the product obtained (90% yield) from $NaBH_4$ (80 mg) reduction of psilostachyin [**2**] (100 mg) in absolute MeOH (5 ml) (11).

(11R)-11,13-Dihydropsilostachyin [**7**].—A solution of **2** (100 mg) in 20 ml of EtOH was hydrogenated over 10 mg of 10% Pd-C for 45 min. After the usual work-up, the two products were separated by preparative tlc ($CHCl_3$-Me_2CO, 7:3). The less polar was identified as isopsilostachyin [**11**], 40 mg (12). The more polar product **7** was recrystallized from Me_2CO/iPr_2O, mp 225-226° (49 mg) and was identical with the natural product in all respects.

Epimerization of **7**.—A solution of **7** (50 mg) and K_2CO_3 (70 mg) in 5 ml of MeOH-H_2O (3:1) was maintained for 1 h in a steam bath. Usual work-up afforded 35 mg of a solid mp 225-227° identical with **13** in all respects.

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